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(54) Title: 3–METHYLIDENYL–2–INDOLINONE MODULATORS OF PROTEIN KINASE

(57) Abstract

The present invention relates to novel 3–methylidenyl–2–indolinone compounds and physiologically acceptable salts and prodrugs thereof which modulate the activity of protein kinases and therefore are expected to be useful in the prevention and treatment of protein kinase related cellular disorders such as cancer.

DESCRIPTION

5   3-METHYLIDENYL-2-INDOLINONE MODLATORS OF PROTEIN KINASE

INTRODUCTION

The present invention relates generally to organic
chemistry, biochemistry, pharmacology and medicine. More

10   particularly, it relates to 3-methylidenyl-2-indolinone
derivatives, and their physiologically acceptable salts and
prodrugs, which modulate the activity of protein kinases
("PKs") and are expected to exhibit a salutary effect against
disorders related to abnormal PK activity.

15

BACKGROUND OF THE INVENTION

The following is offered as background information only
and is not admitted to be prior art to the present invention.

PKs are enzymes that catalyze the phosphorylation of

20   hydroxy groups on tyrosine, serine and threonine residues of
proteins. The consequences of this seemingly simple activity
are staggering; cell growth, differentiation and
proliferation, i.e., virtually all aspects of cell life in one
way or another depend on PK activity. Furthermore, abnormal

25   PK activity has been related to a host of disorders, ranging
from relatively non-life threatening diseases such as
psoriasis to extremely virulent diseases such as glioblastoma
(brain cancer).

The PKs can be conveniently be broken down into two

30   classes, the protein tyrosine kinases (PTKs) and the serine-
threonine kinases (STKs).

One of the prime aspects of PTK activity is their
involvement with growth factor receptors. Growth factor
receptors are cell-surface proteins. When bound by a growth

factor ligand, growth factor receptors are converted to an active form which interacts with proteins on the inner surface of a cell membrane. This leads to phosphorylation on tyrosine residues of the receptor and other proteins and to the

5   formation inside the cell of complexes with a variety of cytoplasmic signaling molecules that, in turn, effect numerous cellular responses such as cell division (proliferation), cell differentiation, cell growth, expression of cellular metabolic processes to the extracellular microenvironment, etc. For a

10 more complete discussion, see Schlessinger and Ullrich, <u>Neuron</u>, 9:303-391 (1992) which is incorporated by reference, including any drawings, as if fully set forth herein.

Growth factor receptors with PTK activity are known as receptor tyrosine kinases ("RTKs"). They comprise a large

15 family of transmembrane receptors with diverse biological activity. At present, at least nineteen (19) distinct subfamilies of RTKs have been identified. An example of these is the subfamily designated the "HER" RTKs, which include EGFR (epithelial growth factor receptor), HER2, HER3 and HER4.

20 These RTKs consist of an extracellular glycosylated ligand binding domain, a transmembrane domain and an intracellular cytoplasmic catalytic domain that can phosphorylate tyrosine residues on proteins.

Another RTK subfamily consists of insulin receptor (IR),

25 insulin-like growth factor I receptor (IGF-1R) and insulin receptor related receptor (IRR). IR and IGF-1R interact with insulin, IGF-I and IGF-II to form a heterotetramer of two entirely extracellular glycosylated α subunits and two β subunits which cross the cell membrane and which contain the

30 tyrosine kinase domain.

A third RTK subfamily is referred to as the platelet derived growth factor receptor ("PDGFR") group, which includes PDGFRα, PDGFRβ, CSFIR, c-kit and c-fms. These receptors

consist of glycosylated extracellular domains composed of variable numbers of immunoglobin-like loops and an intracellular domain wherein the tyrosine kinase domain is interrupted by unrelated amino acid sequences.

5   Another group which, because of its similarity to the PDGFR subfamily, is sometimes subsumed into the later group is the fetus liver kinase ("flk") receptor subfamily. This group is believed to be made of up of kinase insert domain-receptor fetal liver kinase-1 (KDR/FLK-1), flk-1R, flk-4 and fms-like 10 tyrosine kinase 1 (flt-1).

A further member of the tyrosine kinase growth factor receptor family is the fibroblast growth factor ("FGF") receptor subgroup. This group consists of four receptors, FGFR1-4, and seven ligands, FGF1-7. While not yet well 15 defined, it appears that, like the PDGF receptors, the FGF receptors consist of a glycosylated extracellular domain containing a variable number of immunoglobin-like loops and an intracellular domain in which the tyrosine kinase sequence is interrupted by regions of unrelated amino acid sequences.

20   Still another member of the tyrosine kinase growth factor receptor family is the vascular endothelial growth factor (VEGF") receptor subgroup. VEGF is a dimeric glycoprotein similar to PDGF but has different biological functions and target cell specificity in vivo. In particular, VEGF is 25 presently thought to play an essential role is vasculogenesis and angiogenesis.

A more complete listing of the known RTK subfamilies is described in Plowman et al., DN&P, 7(6):334-339 (1994) which is incorporated by reference, including any drawings, as if 30 fully set forth herein.

In addition to the RTKs, there also exists a family of entirely intracellular PTKs called "non-receptor tyrosine kinases" or "cellular tyrosine kinases." This latter designation, abbreviated "CTK," will be used herein. CTKs do

not contain extracellular and transmembrane domains. At present, over 24 CTKs in 11 subfamilies (Src, Frk, Btk, Csk, Abl, Zap70, Fes, Fps, Fak, Jak and Ack) have been identified. The Src subfamily appear so far to be the largest group of

5   CTKs and includes Src, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk. For a more detailed discussion of CTKs, see Bolen, Oncogene, 8:2025-2031 (1993), which is incorporated by reference, including any drawings, as if fully set forth herein.

10   The serine/threonine kinases, STKs, like the CTKs, are predominantly intracellular although there are a few receptor kinases of the STK type. STKs are the most common of the cytosolic kinases; i.e., kinases that perform their function in that part of the cytoplasm other than the cytoplasmic

15   organelles and cytoskelton. The cytosol is the region within the cell where much of the cell's intermediary metabolic and biosynthetic activity occurs; e.g., it is in the cytosol that proteins are synthesized on ribosomes.

RTKs, CTKs and STKs have all been implicated in a host of

20   pathogenic conditions including, significantly, cancer. Other pathogenic conditions which have been associated with PTKs include, without limitation, psoriasis, hepatic cirrhosis, diabetes, angiogenesis, restenosis, ocular diseases, rheumatoid arthritis and other inflammatory disorders,

25   immunological disorders such as autoimmune disease, cardiovascular disease such as atherosclerosis and a variety of renal disorders.

With regard to cancer, two of the major hypotheses advanced to explain the excessive cellular proliferation that

30   drives tumor development relate to functions known to be PK regulated. That is, it has been suggested that malignant cell growth results from a breakdown in the mechanisms that control cell division and/or differentiation. It has been shown that the protein products of a number of proto-oncogenes are

involved in the signal transduction pathways that regulate
cell growth and differentiation. These protein products of
proto-oncogenes include the extracellular growth factors,
transmembrane growth factor PTK receptors (RTKs), cytoplasmic
5   PTKs (CTKs) and cytosolic STKs, discussed above.

In view of the apparent link between PK-related cellular
activities and wide variety of human disorders, it is no
surprise that a great deal of effort is being expended in an
attempt to identify ways to modulate PK activity. Some of
10   these have involved biomimetic approaches using large
molecules patterned on those involved in the actual cellular
processes (e.g., mutant ligands (U.S. App. No. 4,966,849);
soluble receptors and antibodies (App. No. WO 94/10202,
Kendall and Thomas, Proc. Nat'l Acad. Sci., 90:10705-09
15   (1994), Kim, et al., Nature, 362:841-844 (1993)); RNA ligands
(Jelinek, et al., Biochemistry, 33:10450-56); Takano, et al.,
Mol. Bio. Cell 4:358A (1993); Kinsella, et al., Exp. Cell Res.
199:56-62 (1992); Wright, et al., J. Cellular Phys., 152:448-
57) and tyrosine kinase inhibitors (WO 94/03427; WO 92/21660;
20   WO 91/15495; WO 94/14808; U.S. Pat. No. 5,330,992; Mariani, et
al., Proc. Am. Assoc. Cancer Res., 35:2268 (1994)).

In addition to the above, attempts have been made to
identify small molecules which act as PK inhibitors. For
example, bis- monocylic, bicyclic and heterocyclic aryl
25   compounds (PCT WO 92/20642), vinylene-azaindole derivatives
(PCT WO 94/14808) and 1-cyclopropyl-4-pyridylquinolones (U.S.
Pat. No. 5,330,992) have been described as tyrosine kinase
inhibitors. Styryl compounds (U.S. Pat. No. 5,217,999),
styryl-substituted pyridyl compounds (U.S. Pat. No.
30   5,302,606), quinazoline derivatives (EP App. No. 0 566 266
A1), selenaindoles and selenides (PCT WO 94/03427), tricyclic
polyhydroxylic compounds (PCT WO 92/21660) and
benzylphosphonic acid compounds (PCT WO 91/15495) have all

been described as PTK inhibitors useful in the treatment of cancer.

SUMMARY OF THE INVENTION

Our own efforts to identify small organic molecules which
5   modulate PK activity and which, therefore, are expected to be useful in the treatment and prevention of disorders involving abnormal PK activity, has led us to the discovery of a family of 3-methylidenyl-2-indolinone derivatives and their prodrugs and physiologically acceptable salts, which exhibit PK
10   modulating ability and are thereby expected to have a salutary effect against disorders related to abnormal PK activity; it is these compounds which is the subject of this invention.

Thus, the present invention relates generally to 3-methylidenyl-2-indolinones and their prodrugs and
15   physiologically acceptable salts, which modulate the activity of receptor tyrosine kinases (RTKs), non-receptor protein tyrosine kinases (CTKs) and serine/threonine protein kinases (STKs). In addition, the present invention relates to the preparation and use of pharmaceutical compositions of the
20   disclosed compounds and their physiologically acceptable salts and prodrugs in the treatment or prevention of PK driven disorders such as, by way of example and not limitation, cancer, diabetes, hepatic cirrhosis, cardiovasacular disease such as atherosclerosis, angiogenesis, immunological disease
25   such as autoimmune disease and renal disease.

The terms "2-indolinone," indolin-2-one and "2-oxindole" are used interchangeably herein to refer to a molecule having the chemical structure:
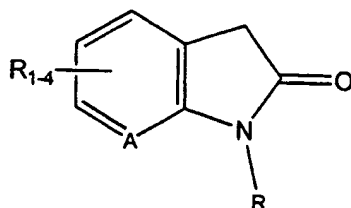
where A is carbon or nitrogen.

A "3-methylidenyl-2-indolinone" refers to a molecule having the chemical structure:

$$R_{1-4} \quad \text{(3-methylidenyl-2-indolinone structure with H, A, N, O, R substituents)}$$
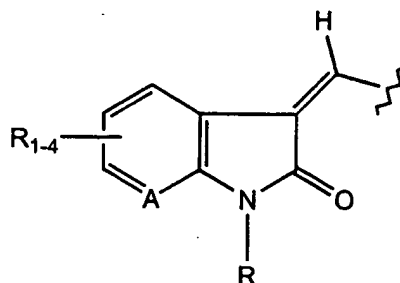
5

where, again, A can be carbon or nitrogen.

A "pharmaceutical composition" refers to a mixture of one or more of the compounds described herein, or physiologically acceptable salts or prodrugs thereof, with other chemical

10   components, such as physiologically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

A "prodrug" refers to an agent which is converted into the parent drug in vivo. Prodrugs are often useful because,

15   in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a

20   prodrug would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the

25   cell where water solubility is beneficial.

A further example of a prodrug might be a short polypeptide, for example, without limitation, a 2 - 10 amino acid polypeptide, bonded through a terminal amino group to a

carboxy group of a compound of this invention wherein the polypeptide is hydrolyzed or metabolized _in vivo_ to release the active molecule.

As used herein, a "physiologically acceptable carrier" or a "pharmaceutically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

A "carrier" refers to a chemical compound which faciliates the incorporation of a compound of interest into cells and tissues. An example, without limitation, of a carrier is dimethyl sulfoxide (DMSO).

A "diluent" refers to a chemical compound, usually a liquid, which dissolves or disperses a compound of interest thereby reducing the concentration of the compound to less than that of the compound alone.

An "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

1.   CHEMISTRY

A.   General Structural Features.

In one aspect, the the present invention relate to 2-indolinones having chemical structure $\underline{1}$:
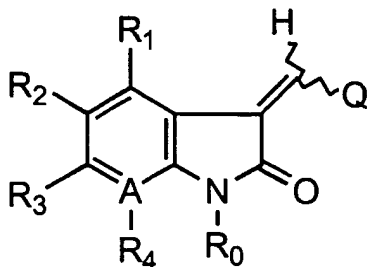
wherein:

A is selected from the group consisting of carbon and
nitrogen;

Q is selected from the group consisting of.
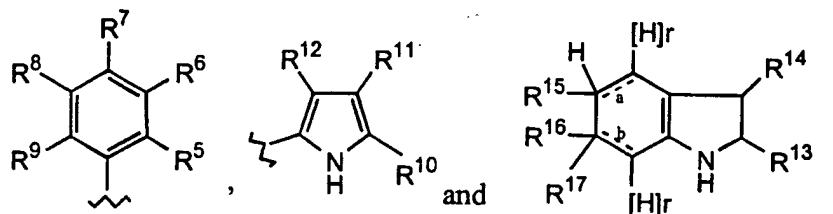
bonds a and b may be either single or double bonds as
indicated by the dotted lines with the proviso that a and b
are both single bonds or both double bonds in any one compound
of this invention;

r is 1 when a and b are double bonds;

r is 2 when a and b are single bonds;

R^0 is selected from the group consisting of hydrogen, alkyl,
$-C(O)OR^{19}$ and $-C(O)R^{19}$;

R^1 is selected from the group consisting of hydrogen, alkyl,
aryl, alkoxy, halo, $-C(O)OR^{19}$, $-(CH_2)_nOC(O)R^{19}$, $-C(O)NR^{19}$ and
$(CH_2)_nR^{20}$, wherein:

R^{19} is selected from the group consisting of hydrogen,
alkyl, alkenyl, alkynyl, cycloalkyl and aryl;

n is 1, 2, 3 or 4;

R^{20} is selected from the group consisting of hydroxy, halo, $-OC(O)NR^{21}R^{22}$; $-OC(S)NR^{21}R^{22}$; $-OC(O)NHSO_2R^{21}$, $-C(O)OR^{19}$, $-NR^{21}R^{22}$ and a heteroalicylic group containing at least one

5   nitrogen atom in the ring, the ring being bonded to the adjacent CH_2 group through the nitrogen atom;

R^2 is selected from the group consisting of hydrogen, alkyl, trihalomethyl, aryl, heteroaryl, heteroalicyclic, alkoxy,

10   halo, $-(CH_2)_nR^{20}$, $-SO_2NR^{21}R^{22}$, $-C(O)OR^{19}$, $-C(O)R^{19}$, $-NHC(O)OR^{19}$, $NHC(O)R^{19}$, $-C(O)(CH_2)_nR^{20}$, $-NR^{21}R^{22}$ and $-N=CR^{23}$ wherein

R^{21} and R^{22} are independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl,

15   cycloalkyl, aryl, heteroaryl and heteroalicyclic;

R^{23} is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl;

20   R^3 is selected from the group consisting of hydrogen, alkyl, trihalomethyl, alkoxy, aryl, aryloxy, heteroaryl, heteroalicyclic, hydroxy, halo, $-SO_2NR^{21}R^{22}$, $-NHSO_2R^{19}$, $-C(O)OR^{19}$, $-NR^{21}R^{22}$ and $-NHC(O)R^{24}$, wherein

25   R^{24} is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl and heteroalicyclic;

R^4 is selected from the group consisting of hydrogen, alkyl, alkoxy and halo;

30

R^5 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, halo, aryl and heteroaryl;

R^6 is selected from the group consisting of hydrogen, alkyl, alkoxy, halo, cycloalkyl, aryl and, combined with R^{18}, a heteroalicyclic group having the structure

wherein

5

    y and y' are independently selected from the group consisting of hydrogen, alkyl and aryl;

R^7 is OR^{18}, wherein R^{18} is selected from the group consisting of

10   alkyl, $-(CH)_nR^{20}$ and, combined with R^6 or R^8, a heteroalicyclic group having the structure

R^8 is selected from the group consisting of hydrogen, alkyl,

15   cycloalkyl, alkoxy, halo, aryl, heteroaryl and, combined with R^{18}, a heteroalicyclic ring having the structure

R^9 is selected from the group consisting of hydrogen, alkyl, alkoxy, halo and $-NR^{21}R^{22}$;

5　R^{10} is selected from the group consisting of alkyl and $-C(O)OR^{19}$;

R^{11} is selected from the group consisting of hydrogen, alkyl and $-C(O)OR^{19}$;

10　R^{12} is $-(CH_2)_nR^{20}$;

R^{13} is either a bond through which Q is bonded to the rest of the molecule or, when it is not, a group selected from the group consisting of hydrogen and alkyl;

15

R^{14} is either a bond through which Q is bonded to the rest of the molecule or, when it is not, a group selected from the group consisting of hydrogen, alkyl and $-(CH_2)_nR^{20}$;

20　R^{15} is either a bond through which Q is bonded to the rest of the molecule or, when it is not, hydrogen;

with the proviso that only one of R^{13}, R^{14} or R^{15} is the bond linking Q to the rest of the molecule in any one
25　compound herein;

R^{16} and R^{17}, when a and b are single bonds, are independently selected from the group consisting of hydrogen, alkyl and aryl and, when a and b are double bonds, R^{16} is hydrogen and R^{17} does
30　not exist; and,

physiologically acceptable salts and prodrugs thereof.

As used herein, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched

chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms (whenever a numerical range; e.g. "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, 5   etc. up to and including 20 carbon atoms). More preferably, it is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, it is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or 10   more independently selected from the group consisting of halo hydroxy, unsubstituted lower alkoxy, aryl optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more halo, hydroxy, 15   unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member 20   heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5- or 6-member heteroalicyclic group 25   having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitrogen (if present) atoms in the group being optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, mercapto, 30   (unsubstituted lower alkyl)thio, arylthio optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-35   sulfonamido, $R^{25}S(O)-$, $R^{25}S(O)_2-$, $-C(O)OR^{25}$, $R^{25}C(O)O-$, and

$-NR^{25}R^{26}$, wherein R^{25} and R^{26} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, unsubstituted (C_3-C_6)cycloalkyl, unsubstituted lower alkenyl, unsubstituted lower alkynyl and aryl optionally

5   optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups.

A "cycloalkyl" group refers to a 3 to 8 member all-carbon monocyclic ring, an all-carbon 5-member/6-member or 6-

10   member/6-member fused bicyclic ring or a multicyclic fused ring(a "fused" ring system means that each ring in the system shares an adjacent pair of carbon atoms with each other ring in the system)group wherein one or more of the rings may contain one or more double bonds but none of the rings has a

15   completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, adamantane, cycloheptane and, cycloheptatriene. A cycloalkyl group may be substituted or

20   unsubstituted. When substituted, the substituent group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, aryl optionally substituted with one or more halo, hydroxy, unsubstituted

25   lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally

30   substituted with one or more halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or

more halo, hydroxy, unsubstituted lower alkyl or unsubstituted
lower alkoxy groups, 5- or 6-member heteroalicyclic group
having from 1 to 3 heteroatoms selected from the group
consisting of nitrogen, oxygen and sulfur, the carbon and

5   nitogen (if present)atoms in the group being optionally
substituted with one or more halo, hydroxy, unsubstituted
lower alkyl or unsubstituted lower alkoxy groups,
mercapto, (unsubstituted lower alkyl)thio, arylthio optionally
substituted with one or more halo, hydroxy, unsubstituted

10 lower alkyl or unsubstituted lower alkoxy groups, cyano, acyl,
thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-
thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-
sulfonamido, $R^{25}S(O)-$, $R^{25}S(O)_2-$, $-C(O)OR^{25}$, $R^{25}C(O)O-$, and $-NR^{25}R^{26}$
are as defined above.

15   An "alkenyl" group refers to an alkyl group, as defined
herein, consisting of at least two carbon atoms and at least
one carbon-carbon double bond.

        An "alkynyl" group refers to an alkyl group, as defined
herein, consisting of at least two carbon atoms and at least

20 one carbon-carbon triple bond.

        An "aryl" group refers to an all-carbon monocyclic or
fused-ring polycyclic (i.e., rings which share adjacent pairs
of carbon atoms) groups having a completely conjugated pi-
electron system. Examples, without limitation, of aryl groups

25 are phenyl, naphthalenyl and anthracenyl. The aryl group may
be substituted or unsubstituted. When substituted, the
substituted group(s) is preferably one or more independently
selected from the group consisting of unsubstituted lower
alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower

30 alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl,
thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-
thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-
sulfonamido, $R^{25}S(O)-$, $R^{25}S(O)_2-$, $-C(O)OR^{25}$, $R^{25}C(O)O-$, and
$-NR^{25}R^{26}$, with R^{25} and R^{26} as defined above.

As used herein, a "heteroaryl" group refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms selected from the group consisting of nitrogen, oxygen and

5   sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups are pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline, purine and carbazole. The heteroaryl group may

10 be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl,

15 thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{25}S(O)-$, $R^{25}S(O)_2-$, $-C(O)OR^{25}$, $R^{25}C(O)O-$, and $-NR^{25}R^{26}$, with R^{25} and R^{26} as defined above.

A "heteroalicyclic" group refers to a monocyclic or fused

20 ring group having in the ring(s) one or more atoms selected from the group consisting of nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic ring may be substituted or unsubstituted.

25 When substituted, the substituted group(s) is preferably one or more independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-

30 thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, $R^{25}S(O)-$, $R^{25}S(O)_2-$, $-C(O)OR^{25}$, $R^{25}C(O)O-$, and $-NR^{25}R^{26}$, with R^{25} and R^{26} as defined above.

A "hydroxy" group refers to an -OH group.

An "alkoxy" group refers to both an -O-(unsubstituted

35 alkyl) and an -O-(unsubstituted cycloalkyl) group.

An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "mercapto" group refers to an -SH group.

A "alkylthio" group refers to both an S-(unsubstituted alkyl) and an -S-(unsubstituted cycloalkyl) group.

A "arylthio" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

An "acyl" group refers to a -C(O)-R" group, where R" is selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, unsubstituted cycloalkyl, aryl optionally substituted with one or more unsubstituted lower alkyl, trihalomethyl, unsubstituted lower alkoxy, halo and $-NR^{25}R^{26}$ groups, heteroaryl (bonded through a ring carbon) optionally substituted with one or more unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR^{25}R^{26}$ groups and heteroalicyclic (bonded through a ring carbon) optionally substituted with one or more unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR^{25}R^{26}$ groups.

An "aldehyde" group refers to an acyl group in which R" is hydrogen.

A "thioacyl" group refers to a -C(S)-R" group, with R" as defined herein.

An "ester" group refers to a -C(O)O-R" group with R" as defined herein except that R" cannot be hydrogen.

An "acetyl" group refers to a -C(O)CH₃ group.

A "halo" group refers to fluorine, chlorine, bromine or iodine.

A "trihalomethyl" group refers to a -CX₃ group wherein X is a halo group as defined herein.

A "trihalomethanesulfonyl" group refers to a X₃CS(=O)₂- groups with X as defined above.

A "cyano" group refers to a -C≡N group.

A "methylenedioxy" group refers to a -OCH₂O- group where

the two oxygen atoms are bonded to adjacent carbon atoms.

An "ethylenedioxy" group refers to a $-OCH_2CH_2O-$ where the two oxygen atoms are bonded to adjacent carbon atoms.

An "O-carbamyl" group refers to a $-OC(O)NR^{25}R^{26}$ group with R[25] and R[26] as defined herein.

An "N-carbamyl" group refers to an $R^{25}OC(O)NR^{26}-$ group, with R[25] and R[26] as defined herein.

An "O-thiocarbamyl" group refers to a $-OC(S)NR^{25}R^{26}$ group with R[25] and R[26] as defined herein.

An "N-thiocarbamyl" group refers to a $R^{25}OC(S)NR^{26}-$ group, with R[25] and R[26] as defined herein.

An "amino" group refers to an $-NR^{25}R^{26}$ group, wherein R[25] and R[26] are both hydrogen.

A "C-amido" group refers to a $-C(O)NR^{25}R^{26}$ group with R[25] and R[26] as defined herein.

An "N-amido" group refers to a $R^{25}C(O)NR^{26}-$ group, with R[25] and R[26] as defined herein.

A "nitro" group refers to a $-NO_2$ group.

B.   Preferred Structural Features.

A presently preferred aspect of this invention is a compound in which Q is

Another presently preferred aspect of this invention is a compound in which Q is

$$R^8 \quad R^7 \quad R^6$$
$$R^9 \qquad R^5$$
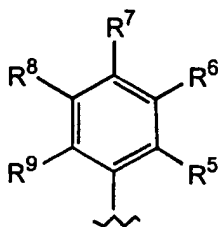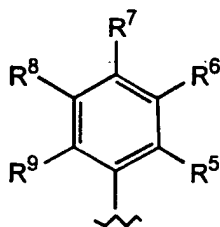
and

R^1 is selected from the group consisting of hydrogen, lower alkyl and $-C(O)OH$;

5   R^2 is selected from the group consisting of hydrogen, lower alkyl, halo, $-C(O)R^{19}$, $-C(O)OR^{19}$, lower alkoxy, $-NR^{21}R^{22}$, $-(CH_2)_nR^{20}$ and $-NHC(O)OR^{19}$;

R^3 is selected from the group consisting of hydrogen, hydroxy, halo, $-NHC(O)O(\text{lower alkyl})$, $-NHSO_2R^{19}$, $-NHC(O)R^{24}$,

10   trihalomethyl, and aryl optionally substituted with one or more lower alkoxy groups;

R^4 is hydrogen;

R^{19} is selected from the group consisting of hydrogen and lower alkyl;

15   n is 2 or 3;

R^{20} is selected from the group consisting of hydroxy, $-C(O)OH$, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl, pyrrolidin-1-yl and $-NR^{21}R^{22}$;

R^{21} and R^{22} are independently selected from the group

20   consisting of hydrogen and lower alkyl; and,

R^{24} is selected from the group consisting of hydrogen and lower alkyl.

It is likewise a presently preferred embodiment of this invention that Q is

R^1 is selected from the group consisting of hydrogen, lower alkyl and -C(O)OH;

R^2 is selected from the group consisting of hydrogen, lower
5   alkyl, halo, -C(O)R^{19}, -C(O)OR^{19}, lower alkoxy, -NR^{21}R^{22},
- (CH$_2$)$_n$R^{20} and -NHC(O)OR^{19};

R^3 is selected from the group consisting of hydrogen, hydroxy,
halo, -NHC(O)O(lower alkyl), -NHSO$_2$R^{19}, -NHC(O)R^{24},
trihalomethyl, and aryl optionally substituted with one or
10   more lower alkoxy groups;

R^4 is hydrogen;

> R^{19} is selected from the group consisting of hydrogen and
> lower alkyl;
>
> n is 2 or 3;
>
15   R^{20} is selected from the group consisting of hydroxy,
> -C(O)OH, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl,
> pyrrolidin-1-yl and -NR^{21}R^{22};
>
> R^{21} and R^{22} are independently selected from the group
> consisting of hydrogen and lower alkyl;
>
20   R^{24} is selected from the group consisting of hydrogen and
> lower alkyl;

R^5 is selected from the group consisting of hydrogen, lower
alkyl, halo, five-member ring heteroaryl and aryl optionally
substituted with one or more groups selected from the group
25   consisting of lower alkyl, halo, hydroxy, -NR^{21}R^{22} and lower
alkoxy;

R^6 is selected from the group consisting of hydrogen, lower
alkyl, 3 to 7-member cycloalkyl, lower alkoxy, halo, aryl

optionally substituted with one or more groups independently
selected from the group consisting of lower alkyl, halo,
hydroxy, -NR^{21}R^{22} and lower alkoxy and, combined with R^{18}, a
group having the structure

5

wherein y and y' are either both hydrogen or both lower alkyl;

R^7 is -OR^{18} wherein R^{18} is selected from the group consisting of
lower alkyl, -(CH$_2$)$_n$R^{20} and, combined with R^6 or R^8, a group
10 having the structure

R^8 is selected from the group consisting of hydrogen, lower
alkyl, 3 to 6-member ring cycloalkyl, lower alkoxy, halo, aryl
15 optionally substituted with one or more groups selected from
the group consisting of lower alkyl, lower alkoxy, halo,
-NR^{21}R^{22} and -NHC(O)(lower alkyl), five-member heteroaryl having
from 1 to 3 heteroatoms in the ring and 6-member heteroaryl
having from 1 to 3 heteroatoms in the ring and, combined with
20 R^{18} a group having the structure
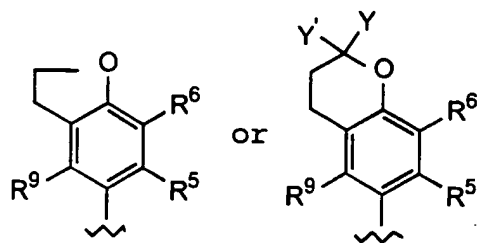
R^9 is selected from the group consisting of hydrogen, lower

5   alkyl; hydroxy, lower alkoxy, halo and -NR^{21}R^{22}.

A further presently preferred aspect of this invention is a compound in which Q is
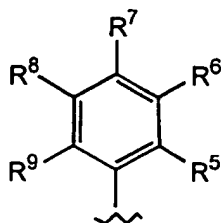
R^1 is selected from the group consisting of hydrogen, lower

10   alkyl and -C(O)OH;

R^2 is selected from the group consisting of hydrogen, lower alkyl, halo, -C(O)R^{19}, -C(O)OR^{19}, lower alkoxy, -NR^{21}R^{22}, -(CH$_2$)$_n$R^{20} and -NHC(O)OR^{19};

R^3 is selected from the group consisting of hydrogen, hydroxy,

15   halo, -NHC(O)O(lower alkyl), -NHSO$_2$R^{19}, -NHC(O)R^{24}, trihalomethyl, and aryl optionally substituted with one or more lower alkoxy groups;

R^4 is hydrogen;

R^{19} is selected from the group consisting of hydrogen and
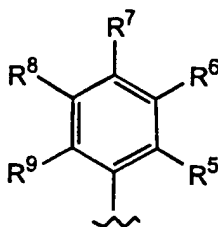
20   lower alkyl;

n is 2 or 3;

R^{20} is selected from the group consisting of hydroxy, -C(O)OH, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl, pyrrolidin-1-yl and -NR^{21}R^{22};

R^{21} and R^{22} are independently selected from the group
consisting of hydrogen and lower alkyl;

R^{24} is selected from the group consisting of hydrogen and
lower alkyl;

R^5 is selected from the group consisting of hydrogen, lower
alkyl, thien-2-yl, thien-3-yl and aryl optionally substituted
with one or more lower alkoxy groups;

R^6 is selected from the group consisting of hydrogen, lower
alkyl, 5 or 6-member cycloalkyl, aryl optionally substituted
with one or more lower alkoxy groups and, combined with R^{18}, a
group having the structure

wherein y and y' are either both hydrogen or both lower
alkyl;

R^7 is $-OR^{18}$, wherein R^{18} is selected from the group consisting
of lower alkyl, $-(CH_2)_nR^{20}$ and, combined with R^6 or R^8, a group
having the structure

n is 2 or 3;

R^{20} is selected from the group consisting of hydroxy,
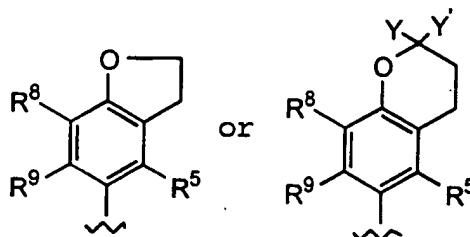$-C(O)OH$, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl

pyrrolidin-1-yl and $-NR^{21}R^{22}$;

R^8 is selected from the group consisting of hydrogen, lower alkyl, 5 or 6-member ring cycloalkyl, lower alkoxy, aryl optionally substituted with a $-NHC(O)$ (lower alkyl) group,

5   thien-2-yl, thien-3-yl, pyridin-2-yl, pyridin-3-yl and, combined with R^{18}, a group having the structure
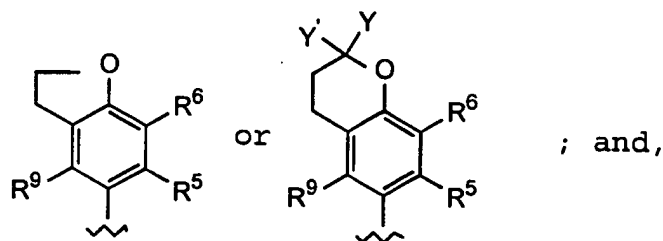
R^9 is selected from the group consisting of hydrogen and lower

10   alkyl.

Still another presently preferred aspect of this invention is a compound in which Q is:

15
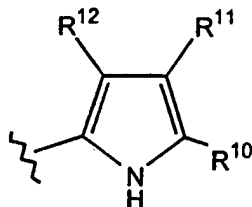
It is a presently preferred aspect of this compound that, in a compound in which Q is
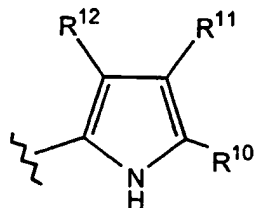
R^1 is selected from the group consisting of hydrogen, lower

alkyl and $-(CH_2)_nR^{20}$;

    n is 2 or 3;

    R^{20} is selected from the group consisting of hydroxy and
-C(O)OH;

5   R^2 is selected from the group consisting of hydrogen, halo,
lower alkyl, lower alkoxy, $-SO_2NR^{21}R^{22}$ and -C(O)OH;

R^3 is selected from the group consisting of hydrogen, halo,
lower alkyl, lower alkoxy and aryl optionally substituted with
one or more lower alkoxy groups; and,

10 R^4 is hydrogen.

    Likewise, in a compound in which Q is

it is a presently preferred aspect of this invention that

15 R^1 is selected from the group consisting of hydrogen, lower
alkyl and $-(CH_2)_nR^{20}$;

    n is 2 or 3;

    R^{20} is selected from the group consisting of hydroxy and
-C(O)OH;

20 R^2 is selected from the group consisting of hydrogen, halo,
lower alkyl, lower alkoxy, $-SO_2NR^{21}R^{22}$ and -C(O)OH;

R^3 is selected from the group consisting of hydrogen, halo,
lower alkyl, lower alkoxy and aryl optionally substituted with
one or more lower alkoxy groups;

25 R^4 is hydrogen;

R^{10} is selected from the group consisting of lower alkyl and
-C(O)OR^{19};

R^{11} is selected from the group consisting of hydrogen, lower
alkyl and $-C(O)OR^{19}$;

R^{12} is $-(CH_2)_nR^{20}$;

R^{19} is selected from the group consisting of hydrogen and lower alkyl;

n is 2 or 3; and,

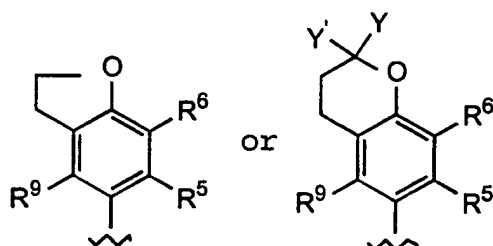R^{20} is selected from the group consisting of hydroxy, -C(O)OH, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl, pyrrolidin-1-yl and $-NR^{21}R^{22}$.

It is yet another presently preferred aspect of this invention that, in a compound in which Q is

R^1 is selected from the group consisting of hydrogen, lower alkyl and $-(CH_2)_nR^{20}$;

n is 2 or 3;

R^{20} is selected from the group consisting of hydroxy and -C(O)OH;

R^2 is selected from the group consisting of hydrogen, halo, lower alkyl, lower alkoxy, $-SO_2NR^{21}R^{22}$ and -C(O)OH;

R^3 is selected from the group consisting of hydrogen, halo, lower alkyl, lower alkoxy and aryl optionally substituted with one or more lower alkoxy groups;

R^4 is hydrogen;

R^{10} is selected from the group consisting of lower alkyl and -C(O)O(lower alkyl);

R^{11} is selected from the group consisting of hydrogen, lower alkyl and $-C(O)OR^{19}$;

R^{12} is $-(CH_2)_nR^{20}$;

R^{19} is selected from the group consisting of hydrogen and

lower alkyl;

n is 2; and,

R[20] is selected from the group consisting of -C(O)OH,
morpholin-1-yl, piperidin-1-yl, piperazin-1-yl,
pyrrolidin-1-yl and -N(lower alkyl)$_2$.

It is also a presently preferred aspect of this invention
that Q is

R[1] is selected from the group consisting of hydrogen and lower
alkyl;

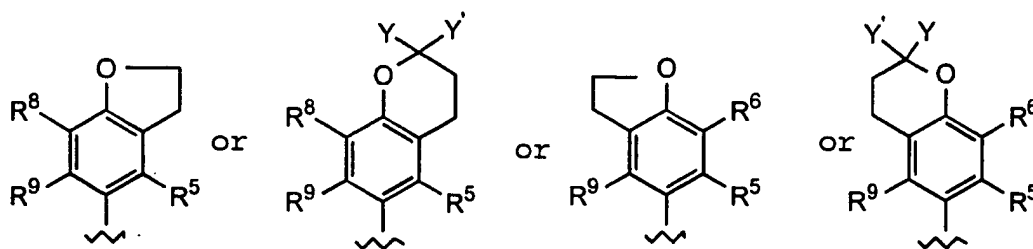R[2] is selected from the group consisting of hydrogen, lower
alkyl, halo, -C(O)OR[19], -C(O)R[19], -NR[21]R[22], -SO$_2$NR[21]R[22] and
-N=C-R[23];

R[3] is selected from the group consisting of hydrogen, halo,
lower alkyl, lower alkoxy, morpholin-4-yl, piperidin-1-yl,
piperazin-1-yl, pyrrolidin-1-yl and aryl optionally
substituted with one or more lower alkoxy groups,; and,
R[4] is hydrogen.

It is a presently preferred aspect of this invention
that, in a compound in which Q is

a and b are both single bonds;

r is 2;

R^1 is selected from the group consisting of hydrogen and lower alkyl;

R^2 is selected from the group consisting of hydrogen, lower
5 alkyl, halo, $-C(O)OR^{19}$, $-C(O)R^{19}$, $-NR^{21}R^{22}$, $-SO_2NR^{21}R^{22}$ and $-N=C-R^{23}$;

R^3 is selected from the group consisting of hydrogen, halo, lower alkyl, lower alkoxy, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl, pyrrolidin-1-yl and aryl optionally
10 substituted with one or more lower alkoxy groups;

R^4 is hydrogen;

R^{13} is a covalent bond through which Q is bonded to the rest of the molecule;

R^{14} is selected from the group consisting of hydrogen, lower
15 alkyl and $-(CH_2)_nR^{20}$;

R^{15} is hydrogen; and,

R^{16} and R^{17} are independently selected from the group consisting of hydrogen and lower alkyl.

It is also a presently preferred aspect of this invention
20 that, in a compound having the substituents indicated in the paragraph immediately above, R^{20} is $-NR^{21}R^{22}$ wherein R^{21} and R^{22} are independently selected from the group consisting of hydrogen and lower alkyl.

It is yet another presently preferred aspect of this
25 invention that, in a compound in which Q is
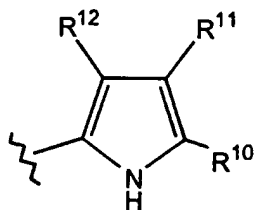

a and b are both double bonds;

r is 1;

R^1 is selected from the group consisting of hydrogen and lower alkyl;

R^2 is selected from the group consisting of hydrogen, lower

5   alkyl, halo, -C(O)OR^{19}, -C(O)R^{19}, -NR^{21}R^{22}, -SO$_2$NR^{21}R^{22} and -N=C-R^{23};

R^3 is selected from the group consisting of hydrogen, halo, lower alkyl, lower alkoxy, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl, pyrrolidin-1-yl and aryl optionally

10 substituted with one or more lower alkoxy groups;

R^4 is hydrogen;

R^{13} is a covalent bond through which Q is bonded to the rest of the molecule;

R^{14} is selected from the group consisting of hydrogen, lower

15 alkyl and -(CH$_2$)$_n$R^{20};

R^{15} and R^{16} are hydrogen; and,

R^{17} does not exist.

A compound in which Q is

20 a and b are both double bonds;

r is 1;

R^1 is selected from the group consisting of hydrogen and lower alkyl;

R^2 is selected from the group consisting of hydrogen, lower

25 alkyl, halo, -C(O)OR^{19}, -C(O)R^{19}, -NR^{21}R^{22}, -SO$_2$NR^{21}R^{22} and -N=C-R^{23};

R^3 is selected from the group consisting of hydrogen, halo, lower alkyl, lower alkoxy, morpholin-4-yl, piperidin-1-yl,

piperazin-1-yl, pyrrolidin-1-yl and aryl optionally
substituted with one or more lower alkoxy groups;

R^4 is hydrogen;

R^{13} is selected from the group consisting of hydrogen and lower
5   alkyl;

R^{14} is a covalent bond through which Q is bonded to the rest of
the molecule;

R^{15} and R^{16} are hydrogen; and,

R^{17} does not exist is another presently preferred aspect of
10   this invention.

It is a presently preferred aspect of this invention
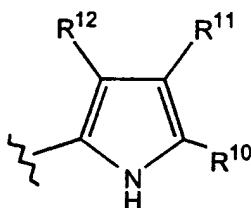that, in a compound where Q is

15

a and b are double bonds;

r is 1;

R^1 is selected from the group consisting of hydrogen and lower
20   alkyl;

R^2 is selected from the group consisting of hydrogen, lower
alkyl, halo, -C(O)OR^{19}, -C(O)R^{19}, -NR^{21}R^{22}, -SO$_2$NR^{21}R^{22} and
-N=C-R^{23};

R^3 is selected from the group consisting of hydrogen, halo,
25   lower alkyl, lower alkoxy, morpholin-4-yl, piperidin-1-yl,
piperazin-1-yl, pyrrolidin-1-yl and aryl optionally
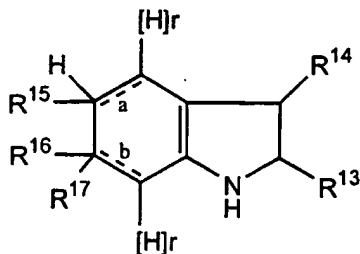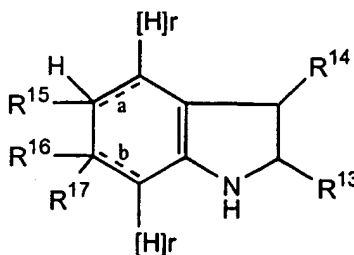substituted with one or more lower alkoxy groups;

R^4 is hydrogen;

R^{13} is selected from the group consisting of hydrogen and lower alkyl;

R^{14} is selected from the group consisting of hydrogen, lower alkyl and $-(CH_2)_nR^{20}$;

R^{15} is a covalent bond through which Q is bonded to the rest of the molecule;

R^{16} are hydrogen; and,

R^{17} does not exist.

A compound selected from the group consisting of:

3-(3,5-Diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

5-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-hydroxy-1,3-dihydroindol-2-one

5-Acetyl-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester

3-(3-Isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one

5-Chloro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one

3-(3-Cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-(3-Cyclopentyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one

3-(3-Cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

5-Bromo-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

5-Chloro-3-(2,3-dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one

5-Chloro-3-(2,2-dimethylchroman-6-ylmethylene)-1,3-
dihydroindol-2-one

N-{3-[3-Cyclohexyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-2-
oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-methoxy-1,3-
dihydroindol-2-one

N-[3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-2-oxo-2,3-dihydro-
1H-indol-6-yl]-acetamide

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-
dihydroindol-2-one

5-Amino-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-
dihydroindol-2-one

5-Chloro-3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-
dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-fluoro-1,3-
dihydroindol-2-one

3-(2,2-Dimethylchroman-6-ylmethylene)-5-fluoro-1,3-
dihydroindol-2-one

5-Chloro-3-[3,5-diisopropyl-4-(2-morpholin-4-ylethoxy)-
benzylidene]-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-7-fluoro-1,3-
dihydroindol-2-one

3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-5-(2-morpholin-4-
ylethyl)-1,3-dihydroindol-2-one

N-[3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-2-oxo-2,3-
dihydro-1H-indol-6-yl]-acetamide

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-ethyl-1,3-
dihydroindol-2-one

N-[2'-Methoxy-5'-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-
biphenyl-3-yl]-acetamide

5-Fluoro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-
dihydroindol-2-one

N-[3-(4-Methoxy-3-thiophen-2-ylbenzylidene)-2-oxo-2,3-dihydro-

1H-indol-6-yl]-acetamide

6-Amino-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-
dihydroindol-2-one

N-[3-(2,2-Dimethylchroman-6-ylmethylene)-2-oxo-2,3-dihydro-1H-
indol-6-yl]-acetamide

5-Bromo-3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-
2-one

3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-1,3-dihydroindol-2-one

5-Bromo-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-
dihydroindol-2-one

5-Fluoro-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-
2-one

3-(3-Isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-
2-one

3-(4,5-Dimethoxy-2-thiophen-2-ylbenzylidene)-1,3-dihydroindol-
2-one

N-{3-[4-(2-Morpholin-4-ylethoxy)-3-thiophen-2-ylbenzylidene]-2-
oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

3-(2,2-Dimethylchroman-6-ylmethylene)-4-methyl-1,3-
dihydroindol-2-one

3-(2,3-Dihydrobenzofuran-5-ylmethylene)-5-fluoro-1,3-
dihydroindol-2-one

3-(3-Cyclohexyl-4-methoxybenzylidene)-5-fluoro-1,3-
dihydroindol-2-one

5-Fluoro-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-
2-one

3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-1,3-
dihydropyrrolo[2,3-b]pyridin-2-one

3-(3'-Ethoxy-6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-
2-one

3-(3-Cyclopentyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-
b]pyridin-2-one

3-(3-Cyclopentyl-4-methoxybenzylidene)-4-methyl-1,3-

dihydroindol-2-one

3-(4,5,2'-Trimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one

N-{3-[4-(2-Morpholin-4-ylethoxy)-3-thiophen-3-ylbenzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

5-Chloro-3-(3-cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-carbamic acid tert-butyl ester

3-(3,5-Diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Bromo-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

N-{3-[3-tert-Butyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

3-(4-Methoxy-3,5-dimethylbenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

5-Bromo-3-[3,5-diisopropyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-1,3-dihydroindol-2-one

3-(3'-Ethoxy-4,5-dimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one

5-Chloro-3-(4-methoxy-3-thiophen-2-ylbenzylidene)-1,3-dihydroindol-2-one

5-Chloro-3-(4-methoxy-3-pyridin-3-ylbenzylidene)-1,3-dihydroindol-2-one

5-Chloro-3-(4,5,3'-trimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one

3-(4,5-Dimethoxy-2-naphthalen-2-ylbenzylidene)-1,3-dihydroindol-2-one

N-[3-(3'-Acetylamino-6-methoxybiphenyl-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide

6-Methoxy-3-(4-methoxy-3-thiophen-3-ylbenzylidene)-1,3-dihydroindol-2-one

3-(6-Methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

3-(2,3-Dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one

5-Chloro-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

3-(3-Cyclohexyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

3-(2,3-dihydrobenzofuran-5-ylmethylene)-4-methyl-1,3-dihydroindol-2-one

3-(3-Isopropyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

3-(6-Methoxybiphenyl-3-ylmethylene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

3-(3-Cyclohexyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

3-(2,3-Dihydrobenzofuran-5-ylmethylene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

5-Bromo-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

5-Bromo-3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

5-Chloro-3-(3-cyclopentyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Chloro-3-(6-methoxybiphenyl-3-ylmethylene)-4-methyl-1,3-dihydroindol-2-one

5-Chloro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Chloro-3-(4-methoxy-3,5-dimethylbenzylidene)-4-methyl-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-trifluoromethyl-1,3-dihydroindol-2-one

6-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-

dihydroindol-2-one

3-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-propionic acid

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-methoxy-1,3-dihydroindol-2-one

5-Butyl-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-(3-methoxyphenyl)-1,3-dihydroindol-2-one

7-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-dihydroindol-2-one

[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-carbamic acid tert-butyl ester

5-Chloro-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

5-Chloro-3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-(6-Methoxybiphenyl-3-ylmethylene)-4-methyl-1,3-dihydroindol-2-one

3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Bromo-3-(2,3-dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one

5-Chloro-3-(3-isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Chloro-3-(2,2-dimethylchroman-6-ylmethylene)-4-methyl-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5,6-dimethoxy-1,3-
dihydroindol-2-one

N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-
1H-indol-6-yl]-methanesulfonamide

N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-
1H-indol-6-yl]-benzamide

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-(3-ethoxyphenyl)-
1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-phenyl-1,3-
dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-fluoro-1,3-
dihydroindol-2-one

5-Fluoro-3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-
dihydroindol-2-one

3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one
is also a preferred embodiment of this invention.

Likewise, a compound selected from the group consisting of:
5-methyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-
dihydroindol-2-one,

3-(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-
indol-5-sulfonic acid amide,

3(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-
indole-5-sulfonic acid methylamide,

3-(3-methyl-1H-indole-2-ylmethylene)-2-oxo-2,3-dihydro-1H-
indole-5-sulfonic acid dimethylamide,

3-(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-
indole-5-carboxylic acid,

5-acetyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-
dihydroindol-2-one,

5-acetyl-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

3-(1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-5-
sulfonic acid amide,

5-amino-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

3-(1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-
carboxylic acid,

6-chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

5-chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

5-bromo-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

3-(1H-indol-2-ylmethylene)-4-methyl-1,3-dihydroindol-2-one,

3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

5-chloro-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-
dihydroindol-2-one,

5-bromo-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-
dihydroindol-2-one,

4-methyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-
dihydroindol-2-one, and

3-(1H-indol-2-ylmethylene)-5[(1H-indol-2-ylmethylene)amino]-
1,3-dihydroindol-2-one

is also a presently preferred embodiment of this invention.

      Finally, with regard to chemical compounds of this
invention, a presently preferred embodiment comprises compounds
selected from the group consisting of:

3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-
tetrahydro-1H-indol-3-yl]-propionic acid,

3-[2-(5-chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,

3-2-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,

3-[2-(4-methyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,

3-[2-(5-methyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,

3-[2-(6-chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,

3-[2-(6-methoxy-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-

4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,

N,N-dimethyl-3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-

4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide,

3-[3-(3-dimethylamino-propyl)-4,5,6,7-tetrahydro-1H-indol-2-

ylmethylene]-1,3-dihydro-indol-2-one,

3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-

tetrahydro-1H-indol-3-yl]-propionamide,

3-[3-(3-morpholin-4-yl-3-oxo-propyl)-4,5,6,7-tetrahydro-1H-

indol-2-ylmethylene]-1,3-dihydro-indol-2-one,

N-methyl-3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-

4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide,

N-(2-morpholin-4-yl-ethyl)-3-[2-(2-oxo-1,2-dihydro-indol-3-

ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-

propionamide, 3-[2-(2-oxo-1,2-dihydro-pyrrolo[2,3-b]pyridin-

3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic

acid,

3-{2-[6-(3-methoxy-phenyl)-2-oxo-1,2-dihydro-indol-3-

ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic

acid,

3-{2-[6-(4-methoxy-phenyl)-2-oxo-1,2-dihydro-indol-3-

ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic

acid,

3-[2-(2-oxo-6-phenyl-1,2-dihydro-indol-3-ylidenemethyl)-

4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,

3-{2-[6-(2-methoxy-phenyl)-2-oxo-1,2-dihydro-indol-3-

ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic

acid,

3-[2-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-

ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic

acid,

3-[2-(6-morpholin-4-yl-2-oxo-1,2-dihydro-indol-3-

ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic

acid,

3-[2-(5-chloro-4-methyl-2-oxo-1,2-dihydro-indol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid,

3-[2-(5-bromo-4-methyl-2-oxo-1,2-dihydro-indol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid,

3-[2-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-N-(2-morpholin-4-yl-
ethyl)-propionamide,

3-[2-(5-chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-N-(2-morpholin-4-yl-
ethyl)-propionamide, and

3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-phenyl]-
propionic acid.

2.   SYNTHESIS/COMBINATORIAL LIBRARIES

An additional aspect of this invention is a combinatorial
library of at least ten 3-methylidenyl-2-indolinone compounds
that can be formed by reacting oxindoles of structure 5 with
5   aldehydes of structure 6, 7 or 8:
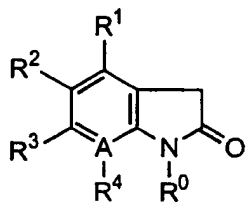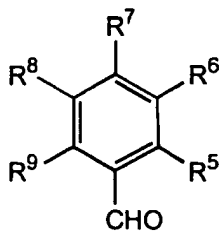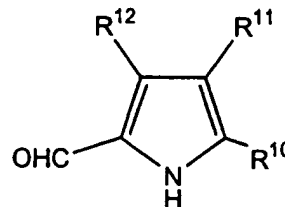
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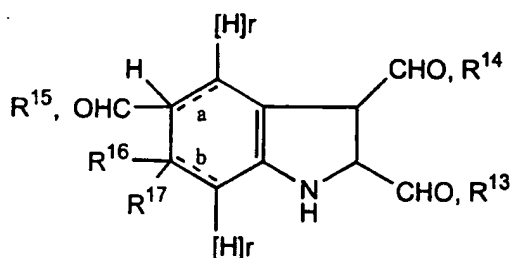
8

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14},
5   R^{15}, R^{16} and R^{17} have the meanings set forth above.

As used herein, a "combinatorial library" refers to all the
compounds formed by the reaction of each compound of one
dimension with a compound in each of the other dimensions in a
multi-dimensional array of compounds. In the context of the
10 present invention, the array is two dimensional and one dimension
represents all the oxindoles of the invention and the second
dimension represents all the aldehydes of the invention. Each
oxindole may be reacted with each and every aldehyde in order to
form a substituted 3-methylidenyl-2-indolinone compound. All
15 substituted 3-methylidenyl-2-indolinone compounds formed in this
way are within the scope of the present invention. Also within
the scope of the present invention are smaller combinatorial
libraries formed by the reaction of some of the oxindoles with
all of the aldehydes, all of the oxindoles with some of the
20 aldehydes, or some of the oxindoles with some of the aldehydes.

The oxindole in the above combinatorial library is
preferably selected from the group consisting of oxindole itself
and substituted oxindoles such as, without limitation, 6-
bromooxindole, 5-hydroxyoxindole, 5-methoxyoxindole, 6-
25 methoxyoxindole, 5-phenylaminosulfonyloxindole, 4-[2-(2-
isopropylphenoxy)ethyl]oxindole, 4-[2-(3-isopropylphenoxy)-
ethyl]oxindole, 4-[2-(4-isopropylphenoxy)ethyl]oxindole, 5-
fluorooxindole, 6-fluorooxindole, 7-fluorooxindole, 6-

trifluoromethyloxindole, 5-chlorooxindole, 6-chlorooxindole, 4-carboxyindole, 5-bromooxindole, 5-bromo-4-methyloxindole, 6-(N-acetamido)oxindole, 4-methyloxindole, 5-methyloxindole, 4-methyl-5-chlorooxindole, 5-ethyloxindole, 6-hydroxyoxindole, 5-acetyloxindole, 5-carboxyindole, 5-aminooxindole, 6-aminooxindole, 4-(2-N-morpholinoethyl)oxindole, 7-azaoxindole, oxindole-4-carabamic acid t-butyl ester, oxindole-6-carbamic acid t-butyl ester, 4-(2-carboxyethyl)oxindole, 5-n-butyloxindole, 5,6-dimethoxyoxindole, 6-(methanesulfonamido)- oxindole, 6-(benzamido)oxindole, 5-ethoxyoxindole, 6-phenyloxindole, 6-(2-methoxyphenyl)oxindole, 6-(3-methoxyphenyl)oxindole, 6-(4-methoxyphenyl)oxindole, 5-aminosulfonyloxindole, 5-isopropyl-aminosulfonyloxindole, 5-dimethylaminosulfonyloxindole, 5-(N-morpholinosulfonyl)oxindole, 4-(2-hydroxyethyl)oxindole, 6-(3-ethoxyphenyl)oxindole, 6-(morpholin-4-yl)oxindole, 5-(2-(N-morpholino)ethyl)oxindole, 5-(methanesulfonamido)oxindole, 5-methoxycarbonyloxindole and 5-carboxyethyloxindole.

The aldehyde in the above combinatorial library is preferably selected from the group consisting of 3-(1-Benzyl-5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)propionic acid, 3-(5-Formyl-1-methoxycarbonylmethyl-2,4-dimethyl-1H-pyrrol-3-yl)propionic acid, 3-(5-Formyl-1,2,4-trimethyl-1H-pyrrol-3-yl)propionic acid, 3-[5-Formyl-1-(3-methoxybenzyl)-2,4-dimethyl-1H-pyrrol-3-yl]propionic acid methyl ester, 3-(1-Cyclohexylmethyl-5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)propionic acid methyl ester, 3-[1-(2,2-Dimethyl-propyl)-5-formyl-2,4-dimethyl-1H-pyrrol-3-yl]propionic acid methyl ester, 1,3,5-Trimethyl-4-(3-morpholin-4-yl-3-oxo-propyl)1H-pyrrole-2-carbaldehyde, 3-(5-Formyl-1,2,4-trimethyl-1H-pyrrol-3-yl)-N-(2-morpholin-4-ylethyl)propionamide, 3-(5-Formyl-1,2,4-trimethyl-1H-pyrrol-3-yl)-N-phenylpropionamide, 1,3,5-trimethyl-4-(3-oxo-3-piperidin-1-ylpropyl)-1H-pyrrole-2-carbaldehyde, 1,3,5-Trimethyl-4-(3-oxo-3-pyrrolidin-1-ylpropyl)-1H-pyrrole-2-carbaldehyde, 3-(5-formyl-1,2,4-trimethyl-1H-pyrrol-3-yl)-N-(4-methoxyphenyl)propionamide, 3-(5-Formyl-1,2,4-trimethyl-1H-pyrrol-3-yl)-N-(4-

methoxyphenyl)propionamide, N-(4-fluorophenyl)-3-(5-formyl-
1,2,4-trimethyl-1H-pyrrol-3-yl)-propionamide, 3-(5-Formyl-1,2,4-
trimethyl-1H-pyrrol-3-yl)-N-(4-trifluoromethylphenyl)-
propionamide, 3-[5-Formyl-1-(3-methoxy-benzyl)-2,4-dimethyl-1H-
5 pyrrol-3-yl]propionic acid, 3-(1-cyclohexylmethyl-5-formyl-2,4-
dimethyl-1H-pyrrol-3-yl)propionic acid, 3-[1-(3-fluorobenzyl)-5-
formyl-2,4-dimethyl-1H-pyrrol-3-yl]propionic acid methyl ester,
3-(1-Benzyl-5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)propionic acid,
3-[1-(4-fluorobenzyl)-5-formyl-2,4-dimethyl-1H-pyrrol-3-
10 yl]propionic acid methyl ester, 3-[1-(4-fluorobenzyl)-5-formyl-
2,4-dimethyl-1H-pyrrol-3-yl]-propionic acid, 3-[1-(3-
fluorobenzyl)-5-formyl-2,4-dimethyl-1H-pyrrol-3-yl]propionic
acid, 3,5-dimethyl-4-(3-morpholin-4-yl-propyl)-1H-pyrrole-2-
carbaldehyde, 4-(3-dimethylaminopropyl)-3,5-dimethyl-1H-pyrrole-
15 2-carbaldehyde, 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic
acid, 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-
2-carbaldehyde, 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic
acid (2-dimethylaminoethyl)amide, 3-(2-formyl-4,5,6,7-
tetrahydro-1H-indol-3-yl)propionic acid, 3-(3-
20 dimethylaminopropyl)-2-formyl-4,5,6,7-tetrahydro-1H-indole,
indole-2-carbaldehyde and 3-methylindole-2-carbaldehyde.

Another aspect of this invention provides a method for the
synthesis of a 3-methylidenyl-2-indolinone of formula 1
comprising reacting an oxindole of formula 5 with an aldehyde of
25 formula 6, 7 or 8 in a solvent, preferably in the presence of a
base.

Examples of the oxindoles of formula 5 which may be reacted
with an aldehyde of formula 6, 7 or 8 to give the substituted 3-
methylidenyl-2-indolinones of formula 1 are oxindole itself and
30 substituted oxindoles such as, without limitation, 6-
bromooxindole, 5-hydroxyoxindole, 5-methoxyoxindole, 6-
methoxyoxindole, 5-phenylaminosulfonyloxindole, 4-[2-(2-
isopropylphenoxy)ethyl]oxindole, 4-[2-(3-isopropylphenoxy)-
ethyl]oxindole, 4-[2-(4-isopropylphenoxy)ethyl]oxindole, 5-
35 fluorooxindole, 6-fluorooxindole, 7-fluorooxindole, 6-

trifluoromethyloxindole, 5-chlorooxindole, 6-chlorooxindole, 4-
carboxyindole, 5-bromooxindole, 5-bromo-4-methyloxindole, 6-(N-
acetamido)oxindole, 4-methyloxindole, 5-methyloxindole, 4-methyl-
5-chlorooxindole, 5-ethyloxindole, 6-hydroxyoxindole, 5-
acetyloxindole, 5-carboxyindole, 5-aminooxindole, 6-
aminooxindole, 4-(2-N-morpholinoethyl)oxindole, 7-azaoxindole,
oxindole-4-carabamic acid t-butyl ester, oxindole-6-carbamic acid
t-butyl ester, 4-(2-carboxyethyl)oxindole, 5-n-butyloxindole, 5,6-
dimethoxyoxindole, 6-(methanesulfonamido)- oxindole, 6-
(benzamido)oxindole, 5-ethoxyoxindole, 6-phenyloxindole, 6-(2-
methoxyphenyl)oxindole, 6-(3-methoxyphenyl)oxindole, 6-(4-
methoxyphenyl)oxindole, 5-aminosulfonyloxindole, 5-isopropyl-
aminosulfonyloxindole, 5-dimethylaminosulfonyloxindole, 5-(N-
morpholinosulfonyl)oxindole, 4-(2-hydroxyethyl)oxindole, 6-(3-
ethoxyphenyl)oxindole, 6-(morpholin-4-yl)oxindole, 5-(2-(N-
morpholino)ethyl)oxindole, 5-(methanesulfonamido)oxindole, 5-
methoxycarbonyloxindole and 5-carboxyethyloxindole.

    Examples of aldehydes of structure 6, 7 or 8 which may be
reacted with oxindoles of structure 2 are, without limitation, 3-
(1-benzyl-5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)propionic acid, 3-
(5-formyl-1-methoxycarbonylmethyl-2,4-dimethyl-1H-pyrrol-3-yl)-
propionic acid, 3-(5-formyl-1,2,4-trimethyl-1H-pyrrol-3-yl)-
propionic acid, 3-[5-formyl-1-(3-methoxybenzyl)-2,4-dimethyl-1H-
pyrrol-3-yl]propionic acid methyl ester, 3-(1-cyclohexylmethyl-5-
formyl-2,4-dimethyl-1H-pyrrol-3-yl)propionic acid methyl ester,
3-[1-(2,2-dimethylpropyl)-5-formyl-2,4-dimethyl-1H-pyrrol-3-yl]-
propionic acid methyl ester, 1,3,5-trimethyl-4-(3-morpholin-4-yl-
3-oxopropyl)-1H-pyrrole-2-carbaldehyde, 3-(5-formyl-1,2,4-
trimethyl-1H-pyrrol-3-yl)-N-(2-morpholin-4-ylethyl)propionamide,
3-(5-formyl-1,2,4-trimethyl-1H-pyrrol-3-yl)-N-phenylpropionamide,
1,3,5-trimethyl-4-(3-oxo-3-piperidin-1-ylpropyl)-1H-pyrrole-2-
carbaldehyde, 1,3,5-trimethyl-4-(3-oxo-3-pyrrolidin-1-yl-propyl)-
1H-pyrrole-2-carbaldehyde, 3-(5-formyl-1,2,4-trimethyl-1H-pyrrol-
3-yl)-N-(4-methoxyphenyl)propionamide, 3-(5-formyl-1,2,4-
trimethyl-1H-pyrrol-3-yl)-N-(4-methoxyphenyl)propionamide, N-(4-

fluorophenyl)-3-(5-formyl-1,2,4-trimethyl-1H-pyrrol-3-yl)-
propionamide, 3-(5-formyl-1,2,4-trimethyl-1H-pyrrol-3-yl)-N-(4-
trifluoromethylphenyl)propionamide, 3-[5-formyl-1-(3-methoxy-
benzyl)-2,4-dimethyl-1H-pyrrol-3-yl]propionic acid, 3-(1-

5   cyclohexylmethyl-5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)propionic
acid, 3-[1-(3-fluorobenzyl)-5-formyl-2,4-dimethyl-1H-pyrrol-3-
yl]propionic acid methyl ester, 3-(1-benzyl-5-formyl-2,4-
dimethyl-1H-pyrrol-3-yl)propionic acid, 3-[1-(4-fluorobenzyl)-5-
formyl-2,4-dimethyl-1H-pyrrol-3-yl]propionic acid methyl ester,

10   3-[1-(4-fluorobenzyl)-5-formyl-2,4-dimethyl-1H-pyrrol-3-yl]-
propionic acid, 3-[1-(3-fluorobenzyl)-5-formyl-2,4-dimethyl-1H-
pyrrol-3-yl]propionic acid, 3,5-dimethyl-4-(3-morpholin-4-yl-
propyl)-1H-pyrrole-2-carbaldehyde, 4-(3-dimethylaminopropyl)-3,5-
dimethyl-1H-pyrrole-2-carbaldehyde, 5-formyl-2,4-dimethyl-1H-

15   pyrrole-3-carboxylic acid, 3,5-dimethyl-4-(4-methylpiperazine-1-
carbonyl)-1H-pyrrole-2-carbaldehyde, 5-formyl-2,4-dimethyl-1H-
pyrrole-3-carboxylic acid (2-dimethylaminoethyl)amide, 3-(2-
formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)propionic acid, 3-(3-
dimethylaminopropyl)-2-formyl-4,5,6,7-tetrahydro-1H-indole,

20   indole-2-carbaldehyde and 3-methylindole-2-carbaldehyde.

The synthesis of an aminopropyl-4,5,6,7-tetrahydro-1H-
indole, structure 9, below, which is an intermediate to some
of the compounds of this invention, is another aspect of this
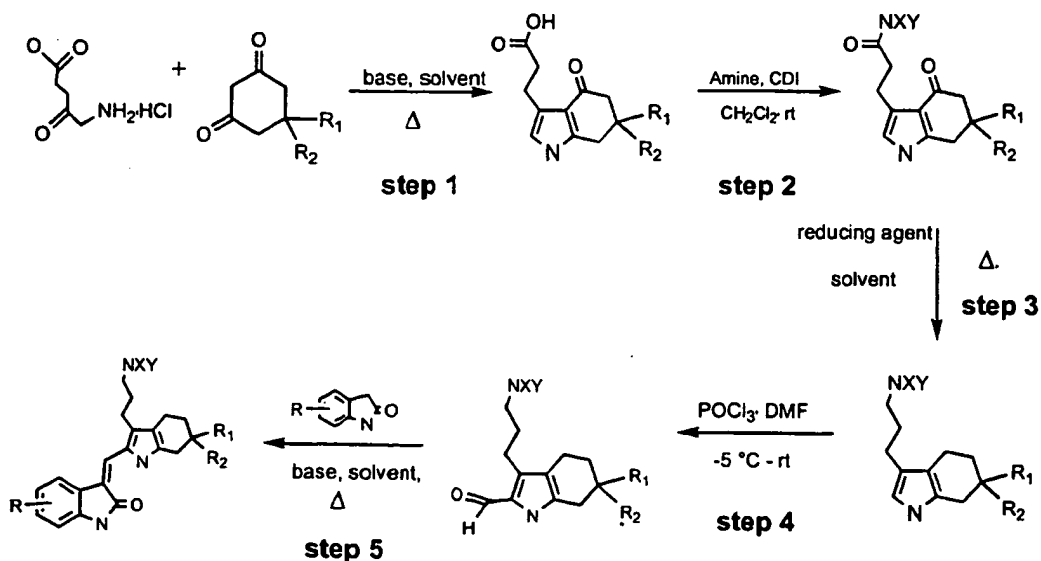
invention:

In Step 1, the base can be an inorganic or an organic base. Examples of inorganic and organic bases are presented elsewhere herein. Preferrably the base is sodium acetate. The "solvent" may be any solvent in which base and the other reactants are sufficiently soluble for reaction to take place. Preferred solvents are polar protic solvents (defined elsewhere herein) such as water, methanol and ethanol. The presently preferred solvent is water. The reaction is carried out at temperatures of from about 60° C to about 180° C. Preferably the temperature is between about 60° C and 180° C, more preferably between 80° C and 150° C, most preferably between 100 and 120° C. The reaction is allowed to run for from 1 to 30 hours, preferably from 2 to 20 hours, most preferably from 4 to 15 hours.

In step 2, a carboxylic acid group is converted to an amide group. Procedures for accomplishing this conversion are well-known in the art. The reaction of an amine with the carboxylic acid in the presence of 1,1'-carbonyldiimidazole is the presently preferred method. Any aprotic solvent may be used; in a presently preferred embodiment the solvent is a non-polar aprotic solvent, in particular dichoromethane. The reaction may be carried out at room temperature or at an elevated temperature up to about 100° C; a presently preferred embodiment is to run the reaction at about room temperature.

Step 3 is the simultaneous reduction of the keto and the amido group using a reducing agent such as, for example and without limitation, lithium aluminum hydride. The reaction is carried out in an aprotic solvent (defined elsewhere herein). A presently preferred solvent is tetrahydrofuran. The reaction is carried out at temperatures from about room temperature to about 80° C, preferrably from about 50° C to

about 70° C, most preferably, the reaction is carried out in tetrahydrofuran at reflux.

Step 4 is the well-known formylation of the aromatic pyrrole ring using phosphorus oxychloride and N,N-dimethylformamide.

Step 5, the condensation of an aldehyde with an oxindole to form a 3-methylidenyl-2-indolinone of this invention is carried out in a solvent which may contain a base. The base may be an organic or an inorganic base. If an organic base is used, preferably it is a nitrogen base. Examples of organic nitrogen bases include, but are not limited to, diisopropylamine, trimethylamine, triethylamine, aniline, pyridine, 1,8-diazabicyclo-[5.4.1]undec-7-ene, pyrrolidine and piperidine.

Examples of inorganic bases are, without limitation, ammonia, alkali metal or alkaline earth hydroxides, phosphates, carbonates, bicarbonates, acetates, bisulfates and amides. The alkali metals include, lithium, sodium and potassium while the alkaline earths include calcium, magnesium and barium.

In a presently preferred embodiment of this invention, when the solvent is a protic solvent, such as water or alcohol, the base is an alkali metal or an alkaline earth inorganic base, preferably, a alkali metal or an alkaline earth hydroxide.

It will be clear to those skilled in the art, based both on known general principles of organic synthesis and on the disclosures herein which base would be most appropriate for the reaction contemplated.

The solvent in which the reaction is carried out may be a protic or an aprotic solvent, preferably it is a protic solvent. A "protic solvent" is a solvent which has hydrogen atom(s) covalently bonded to oxygen or nitrogen atoms which renders the hydrogen atoms appreciably acidic and thus capable of being "shared" with a solute through hydrogen bonding.

Examples of protic solvents include, without limitation, water and alcohols.

An "aprotic solvent" may be polar or non-polar but, in either case, does not contain acidic hydrogens and therefore is not capable of hydrogen bonding with solutes. Examples, without limitation, of non-polar aprotic solvents, are pentane, hexane, benzene, toluene, methylene chloride and carbon tetrachloride.

Examples of polar aprotic solvents are chloroform, tetrahydrofuran, dimethylsulfoxide and dimethylformamide.

In a presently preferred embodiment of this invention, the solvent is a protic solvent, preferably water or an alcohol such as ethanol.
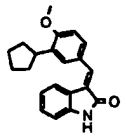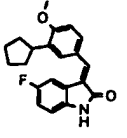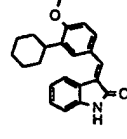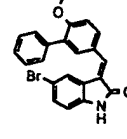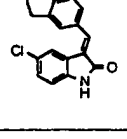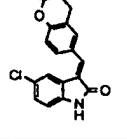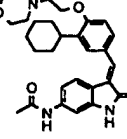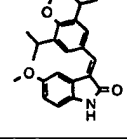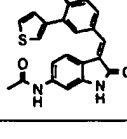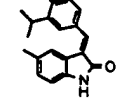
The reaction is carried out at temperatures greater than room temperature. The temperature is generally from about 30° C to about 150° C, preferably about 80°C to about 100° C, most preferable about 75° C to about 85° C, which is about the boiling point of ethanol.
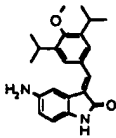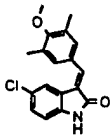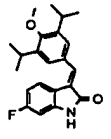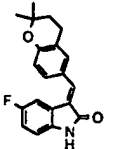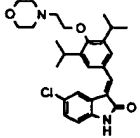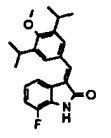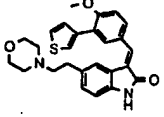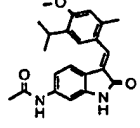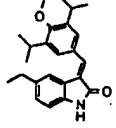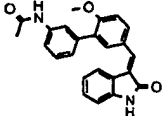
By "about" is meant that a temperature range described herein is preferably within 10 degrees Celcius of the indicated temperature, more preferably within 5 degrees Celcius of the indicated temperature and, most preferably, within 2 degrees Celcius of the indicated temperature. Thus, for example, by "about 75° C" is meant 75° C ± 10° C, preferably 75° C ± 5° C and most preferably, 75° C ± 2° C.

Some representative compounds of this invention are shown in Table 1. The compounds shown are examples only and are not to be construed as limiting the scope of this invention in any manner whatsoever.
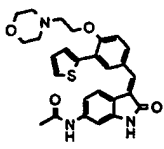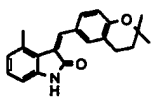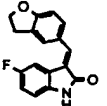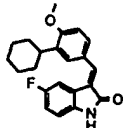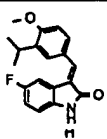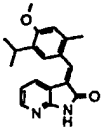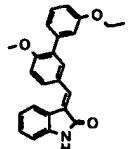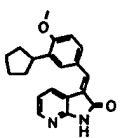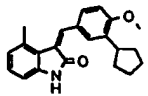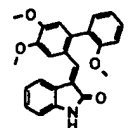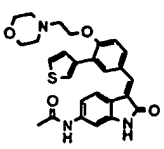
TABLE 1
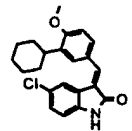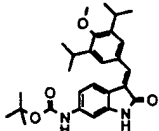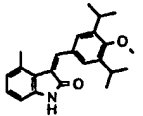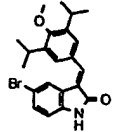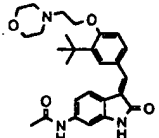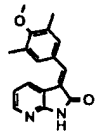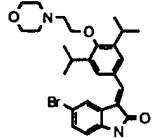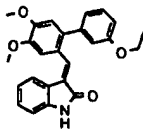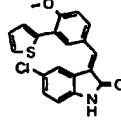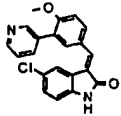
Compound	Structures	Names
1		3-(3,5-Diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
2		5-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
3		N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide
4		3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-hydroxy-1,3-dihydroindol-2-one
5		5-Acetyl-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
6		3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester
7		3-(3-Isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
8		3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one
9		5-Chloro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one

10		3-(3-Cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
11		3-(3-Cyclopentyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one
12		3-(3-Cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
13		5-Bromo-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one
14		5-Chloro-3-(2,3-dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one
15		5-Chloro-3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one
16		N-{3-[3-Cyclohexyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide
17		3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-methoxy-1,3-dihydroindol-2-one
18		N-[3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide
19		3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-dihydroindol-2-one
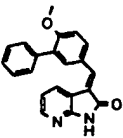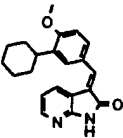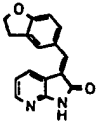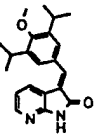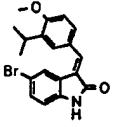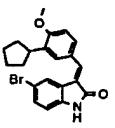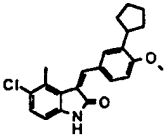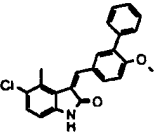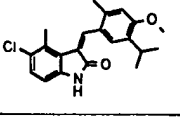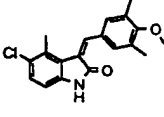
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21		5-Chloro-3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-dihydroindol-2-one
22		3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-fluoro-1,3-dihydroindol-2-one
23		3-(2,2-Dimethylchroman-6-ylmethylene)-5-fluoro-1,3-dihydroindol-2-one
24		5-Chloro-3-[3,5-diisopropyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-1,3-dihydroindol-2-one
25		3-(3,5-Diisopropyl-4-methoxybenzylidene)-7-fluoro-1,3-dihydroindol-2-one
26		3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-5-(2-morpholin-4-ylethyl)-1,3-dihydroindol-2-one
27		N-[3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide
28		3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-ethyl-1,3-dihydroindol-2-one
29		N-[2'-Methoxy-5'-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-biphenyl-3-yl]-acetamide

30		5-Fluoro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one
31		N-[3-(4-Methoxy-3-thiophen-2-ylbenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide
32		6-Amino-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
33		N-[3-(2,2-Dimethylchroman-6-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide
34		5-Bromo-3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol 2-one
35		3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-1,3-dihydroindol-2-one
36		5-Bromo-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one
37		5-Fluoro-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2 one
38		3-(3-Isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one
39		3-(4,5-Dimethoxy-2-thiophen-2-ylbenzylidene)-1,3-dihydroindol-2-one

40		N-{3-[4-(2-Morpholin-4-ylethoxy)-3-thiophen-2-ylbenzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide
41		3-(2,2-Dimethylchroman-6-ylmethylene)-4-methyl-1,3-dihydroindol-2-one
42		3-(2,3-Dihydrobenzofuran-5-ylmethylene)-5-fluoro-1,3-dihydroindol-2-one
43		3-(3-Cyclohexyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one
44		5-Fluoro-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
45		3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one
46		3-(3'-Ethoxy-6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one
47		3-(3-Cyclopentyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one
48		3-(3-Cyclopentyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one
49		3-(4,5,2'-Trimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one
50		N-{3-[4-(2-Morpholin-4-ylethoxy)-3-thiophen-3-ylbenzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide
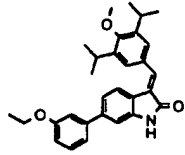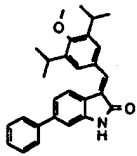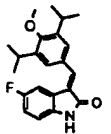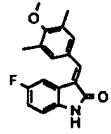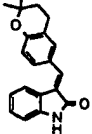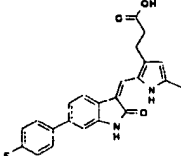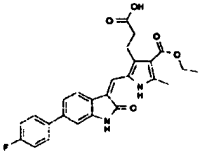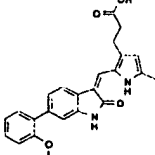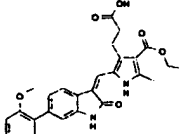
51		5-Chloro-3-(3-cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
52		[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-carbamic acid tert-butyl ester
53		3-(3,5-Diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one
54		5-Bromo-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
55		N-{3-[3-tert-Butyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide
56		3-(4-Methoxy-3,5-dimethylbenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one
57		5-Bromo-3-[3,5-diisopropyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-1,3-dihydroindol-2-one
58		3-(3'-Ethoxy-4,5-dimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one
59		5-Chloro-3-(4-methoxy-3-thiophen-2-ylbenzylidene)-1,3-dihydroindol-2-one
60		5-Chloro-3-(4-methoxy-3-pyridin-3-ylbenzylidene)-1,3-dihydroindol-2-one

61		5-Chloro-3-(4,5,3'-trimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one
62		3-(4,5-Dimethoxy-2-naphthalen-2-ylbenzylidene)-1,3-dihydroindol 2-one
63		N-[3-(3'-Acetylamino-6-methoxybiphenyl-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide
64		6-Methoxy-3-(4-methoxy-3-thiophen-3-ylbenzylidene)-1,3-dihydroindol-2-one
65		3-(6-Methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one
66		3-(2,3-Dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one
67		5-Chloro-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2 one
68		3-(3-Cyclohexyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one
69		3-(2,3-dihydrobenzofuran-5-ylmethylene)-4-methyl-1,3-dihydroindol-2-one
70		3-(3-Isopropyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

71		3-(6-Methoxybiphenyl-3-ylmethylene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one
72		3-(3-Cyclohexyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one
73		3-(2,3-Dihydrobenzofuran-5-ylmethylene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one
74		3-(3,5-Diisopropyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one
75		5-Bromo-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
76		5-Bromo-3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
77		5-Chloro-3-(3-cyclopentyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one
78		5-Chloro-3-(6-methoxybiphenyl-3-ylmethylene)-4-methyl-1,3-dihydroindol-2-one
79		5-Chloro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-4-methyl-1,3-dihydroindol-2-one
80		5-Chloro-3-(4-methoxy-3,5-dimethylbenzylidene)-4-methyl-1,3-dihydroindol-2-one

81		3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-trifluoromethyl-1,3-dihydroindol-2-one
82		6-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
83		3-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-propionic acid
84		3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-methoxy-1,3-dihydroindol-2-one
85		5-Butyl-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
86		3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid
87		3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-(3-methoxyphenyl)-1,3-dihydroindol-2-one
88		7-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-dihydroindol-2-one
89		[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-carbamic acid tert-butyl ester
90		5-Chloro-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
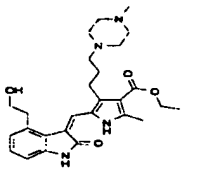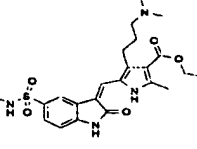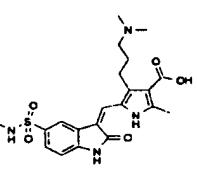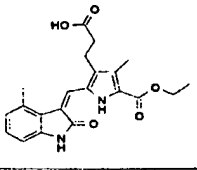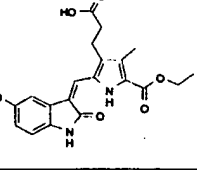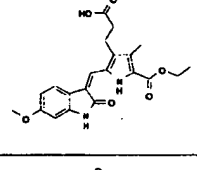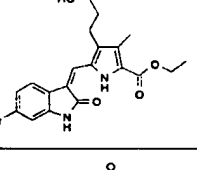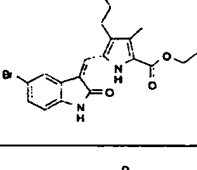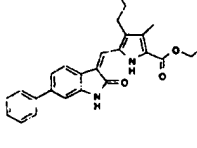
91		5-Chloro-3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
92		3-(6-Methoxybiphenyl-3-ylmethylene)-4-methyl-1,3-dihydroindol-2-one
93		3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-4-methyl-1,3-dihydroindol-2-one
94		5-Bromo-3-(2,3-dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one
95		5-Chloro-3-(3-isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one
96		5-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one
97		5-Chloro-3-(2,2-dimethylchroman-6-ylmethylene)-4-methyl-1,3-dihydroindol-2-one
98		3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid
99		3-(3,5-Diisopropyl-4-methoxybenzylidene)-5,6-dimethoxy-1,3-dihydroindol-2-one
100		N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-methanesulfonamide
101		N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-benzamide
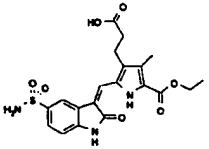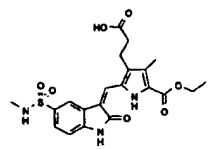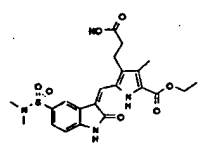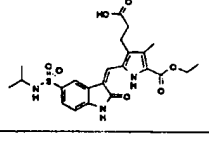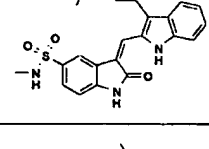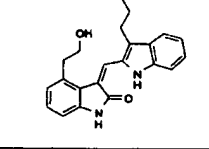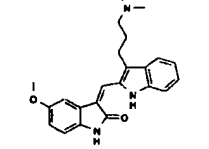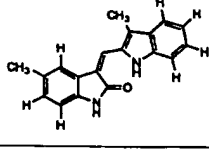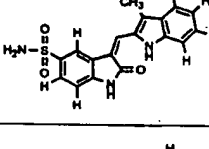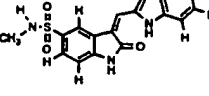
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103		3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-phenyl-1,3-dihydroindol-2-one
104		3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one
105		5-Fluoro-3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-dihydroindol-2-one
106		3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one
107		3-{2-[6-(4-Fluoro-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-5-methyl-1H-pyrrol-3-yl}-propionic acid
108		4-(2-Carboxy-ethyl)-5-[6-(4-fluoro-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester
109		3-{2-[6-(2-Methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-5-methyl-1H-pyrrol-3-yl}-propionic acid
110		4-(2-Carboxy-ethyl)-5-[6-(2-methoxy-phenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester
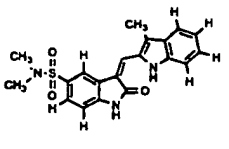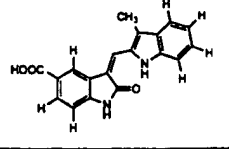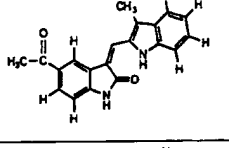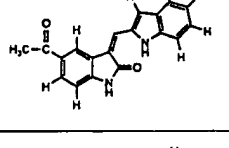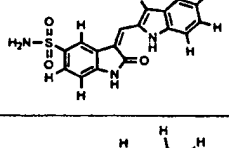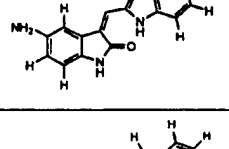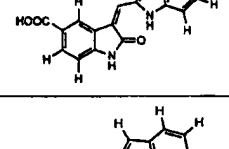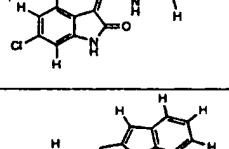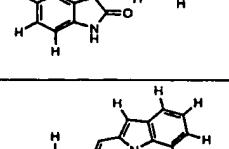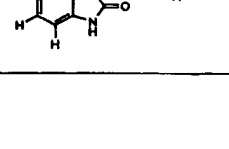
111		3-[2-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1H-pyrrol-3-yl]-propionic acid
112		4-(2-Carboxy-ethyl)-5-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester
113		4-(2-Carboxy-ethyl)-2-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
114		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-(2-carboxy ethyl)-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester
115		3-[5-Methyl-2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid
116		3-[2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1H-pyrrol-3-yl]-propionic acid
117		4-(2-Carboxy-ethyl)-3-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester
118		2-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-(3-morpholin-4-yl-propyl)-1H-pyrrole-3-carboxylic acid ethyl ester
119		2-Methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

120		4-(3-Dimethylamino-propyl)-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester
121		5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-(3-morpholin-4-yl-propyl)-1H-pyrrole-3-carboxylic acid ethyl ester
122		5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-1H-pyrrole-3-carboxylic acid ethyl ester
123		2-Methyl-4-(3-morpholin-4-yl-propyl)-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
124		2-Methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
125		3-[4-Ethoxycarbonyl-5-methyl-3-(3-morpholin-4-yl-propyl)-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid
126		2-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-(3-pyrrolidin-1-yl-propyl)-1H-pyrrole-3-carboxylic acid ethyl ester
127		5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-(3-pyrrolidin-1-yl-propyl)-1H-pyrrole-3-carboxylic acid ethyl ester

128		3-[4-Ethoxycarbonyl-5-methyl-3-(3-pyrrolidin-1-yl-propyl)-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid
129		3-[4-Ethoxycarbonyl-5-methyl-3-[3-(4-methyl-piperazin-1-yl)-propyl]-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid
130		2-Methyl-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-4-(3-pyrrolidin-1-yl-propyl)-1H-pyrrole-3-carboxylic acid ethyl ester
131		5-[4-(2-Hydroxy-ethyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-(3-morpholin-4-yl-propyl)-1H-pyrrole-3-carboxylic acid ethyl ester
132		5-[4-(2-Hydroxy-ethyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-(3-pyrrolidin-1-yl-propyl)-1H-pyrrole-3-carboxylic acid ethyl ester
133		4-(3-Dimethylamino-propyl)-2-methyl-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
134		3-[3-(3-Dimethylamino-propyl)-4-ethoxycarbonyl-5-methyl-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid
135		4-(3-Dimethylamino-propyl)-5-[4-(2-hydroxy-ethyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester

136		5-[4-(2-Hydroxy-ethyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-1H-pyrrole-3-carboxylic acid ethyl ester
137		4-(3-Dimethylamino-propyl)-2-methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
138		4-(3-Dimethylamino-propyl)-2-methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid
139		4-(2-Carboxy-ethyl)-3-methyl-5-(4-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester
140		4-(2-Carboxy-ethyl)-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester
141		4-(2-Carboxy-ethyl)-5-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester
142		5-(6-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-(2-carboxy ethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester
143		5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-(2-carboxy ethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester
144		4-(2-Carboxy-ethyl)-3-methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester
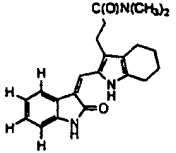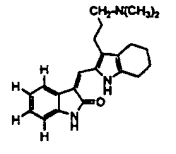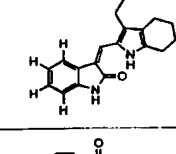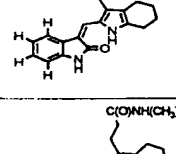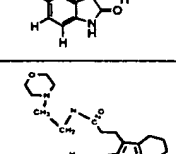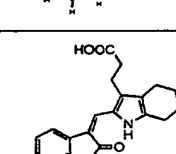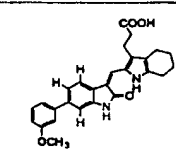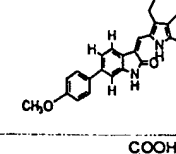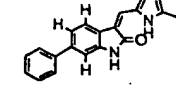
145		4-(2-Carboxy-ethyl)-3-methyl-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester
146		4-(2-Carboxy-ethyl)-3-methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester
147		4-(2-Carboxy-ethyl)-5-(5-dimethylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester
148		4-(2-Carboxy-ethyl)-5-(5-isopropylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester
149		3-[3-(3-Dimethylamino-propyl)-1H-indol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide
150		3-[3-(3-Dimethylamino-propyl)-1H-indol-2-ylmethylene]-4-(2-hydroxy-ethyl)-1,3-dihydroindol-2-one
151		3-[3-(3-Dimethylamino-propyl)-1H-indol-2-ylmethylene]-5-methoxy-1,3-dihydroindol-2-one
152		5-methyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
153		3-(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-5-sulfonic acid amide
154		3(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide

155		3-(3-methyl-1H-indole-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide
156		3(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid
157		5-acetyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
158		5-acetyl-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
159		3-(1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-5-sulfonic acid amide
160		5-amino-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
161		3-(1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid
162		6-chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
163		3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
164		5-chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
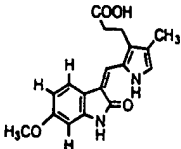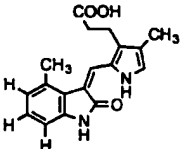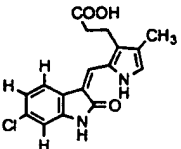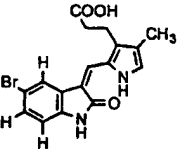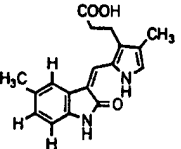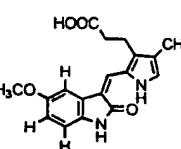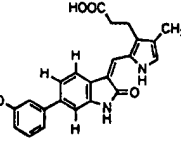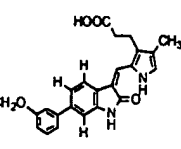
165		5-bromo-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
166		3-(1H-indol-2-ylmethylene)-4-methyl-1,3-dihydroindol-2-one
167		3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
168		5-chloro-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
169		5-bromo-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
170		4-methyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
171		3-(1H-indol-2-ylmethylene)-5[(1H-indol-2-ylmethylene)-amino]-1,3 dihydroindol-2-one
172		3-(1H-Indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid amide
173		3-(1H-Indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide
174		3-(1H-Indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid
175		3-(3-Methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole 5-sulfonic acid amide

176		3-(1H-Indol-5-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide
177		3-(1H-Indol-5-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid
178		3-(1H-Indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide
179		5-Amino-3-(1H-indol-5-ylmethylene)-1,3-dihydroindol-2-one
180		5-Amino-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one
181		3-(2-Methyl-1H-indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole 5-sulfonic acid methylamide
182		3-(2-Methyl-1H-indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole 5-carboxylic acid
183		3-(1H-Indol-5-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide
184		3-(2-Methyl-1H-indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole 5-sulfonic acid dimethylamide
185		3-(1H-Indol-5-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid amide
186		3-(1H-Indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide

187		5-Amino-3-(2-methyl-1H-indol-3-ylmethylene)-1,3-dihydroindol-2-one
188		3-(1H-Indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide
189		3-(2-Methyl-1H-indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole 5-sulfonic acid amide
190		3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
191		3-[2-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
192		3-[2-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
193		3-[2-(4-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
194		3-[2-(5-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
195		3-[2-(6-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
196		3-[2-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

197		N,N-dimethyl-3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide
198		3-[3-(3-dimethylamino-propyl)-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene]-1,3-dihydroindol-2-one
199		3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide
200		3-[3-(3-morpholin-4-yl-3-oxo-propyl)-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene]-1,3-dihydroindol-2-one
201		N-methyl-3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide
202		N-(2-morpholin-4-yl-ethyl)-3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide
203		3-[2-(2-oxo-1,2-dihydro-pyrrolo[2,3-b]pyridin-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
204		3-{2-[6-(3-methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic acid
205		3-{2-[6-(4-methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic acid
206		3-[2-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

207		3-{2-[6-(2-methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic acid
208		3-[2-(5-isopropylaminosulfonyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
209		3-[2-(6-morpholin-4-yl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
210		3-[2-(5-chloro-4-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
211		3-[2-(5-bromo-4-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
212		3-[2-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-N-(2-morpholin-4-yl-ethyl)-propionamide
213		3-[2-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-N-(2-morpholin-4-yl-ethyl)-propionamide
214		3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-phenyl]-propionic acid
215		3-[4-Methyl-2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid
216		3-[2-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid

217		3-[2-(6-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid
218		3-[2-(4-Methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid
219		3-[2-(6-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid
220		3-[2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid
221		3-[2-(5-Methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid
222		3-[2-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid
223		3-{2-[6-(3-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4-methyl-1H-pyrrol-3-yl}-propionic acid
224		3-{2-[6-(3-Ethoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4-methyl-1H-pyrrol-3-yl}-propionic acid

3.   **BIOCHEMISTRY/PHARMACOTHERAPY**

Another aspect of this invention relates to a method for the modulation of the catalytic activity of a PK by contacting a PK with a compound of this invention or a physiologically acceptable salt or prodrug thereof.

As used herein, "PK" refers to receptor protein tyrosine kinase (RTKs), non-receptor or "cellular" tyrosine kinase (CTKs) and serine-threonine kinases (STKs).

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by, practitioners of the chemical, pharmaceutical, biological, biochemical and medical arts.

As used herein, the term "modulation" or "modulating" refers to the alteration of the catalytic activity of RTKs, CTKs and STKs. In particular, modulating refers to the activation of the catalytic activity of RTKs, CTKs and STKs, preferably the activation or inhibition of the catalytic activity of RTKs, CTKs and STKs, depending on the concentration of the compound or salt to which the RTK, CTK or STK is exposed or, more preferably, the inhibition of the catalytic activity of RTKs, CTKs and STKs.

The term "catalytic activity" as used herein refers to the rate of phosphorylation of tyrosine under the influence, direct or indirect, of RTKs and/or CTKs or the phosphorylation of serine and threonine under the influence, direct or indirect, of STKs.

The term "contacting" as used herein refers to bringing a compound of this invention and a target PK together in such a manner that the compound can affect the catalytic activity of the PK, either directly, i.e., by interacting with the kinase itself, or indirectly, i.e., by interacting with another molecule on which the catalytic activity of the kinase is dependent. Such "contacting" can be accomplished "_in vitro_," i.e., in a test

tube, a petri dish or the like. In a test tube, contacting may involve only a compound and a PK of interest or it may involve whole cells. Cells may also be maintained or grown in cell culture dishes and contacted with a compound in that environment.

5   In this context, the ability of a particular compound to affect a PK related disorder, i.e., the IC_{50} of the compound, defined below, can be determined before use of the compounds in vivo with more complex living organisms is attempted. For cells outside the organism, multiple methods exist, and are well-known to those

10   skilled in the art, to get the PKs in contact with the compounds including, but not limited to, direct cell microinjection and numerous transmembrane carrier techniques.

A further aspect of this invention is that the modulation of the catalytic activity of PKs using a compound of this

15   invention may be carried out in vitro or in vivo.

"In vitro" refers to procedures performed in an artificial environment such as, e.g., without limitation, in a test tube or culture medium.

As used herein, "in vivo" refers to procedures performed

20   within a living organism such as, without limitation, a mouse, rat, rabbit ot human being.

A still further aspect of this invention is that the protein kinase whose catalytic activity is being modulated by a compound of this invention is selected from the group

25   consisting of receptor protein tyrosine kinases, a cellular (or non-receptor) tyrosine kinases and serine-threonine kinases.

It is an aspect of this invention that the receptor protein kinase whose catalytic activity is modulated by a

30   compound of this invention is selected from the group consisting of EGF, HER2, HER3, HER4, IR, IGF-1R, IRR, PDGFRα, PDGFRβ, CSFIR, C-Kit, C-fms, Flk-1R, Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R.

In addition, it is an aspect of this invention that the
cellular tyrosine kinase whose catalytic activity is
modulated by a compound of this invention is selected from
the group consisting of Src, Frk, Btk, Csk, Abl, ZAP70,

5   Fes/Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr
and Yrk.

Another aspect of this invention is that the serine-
threonine protein kinase whose catalytic activity is modulated
by a compound of this invention is selected from the group

10 consisting of CDK2 and Raf.

A pharmaceutical composition of a compound of this
invention with a pharmaceutically acceptable carrier or
excipient is yet another aspect of this invention. Such
pharmaceutical composition may contain both carriers and

15 excipients as well as other components generally known to
those skilled in the formulation of pharmaceutical
compositions.

A method for treating or preventing a protein kinase
related disorder in an organism comprising administering a

20 therapeutically effective amount of a compound, salt or
prodrug of this invention to an organism in need of such
treatment is another aspect of this invention.

As used herein, "PK related disorder," "PK driven
disorder," and "abnormal PK activity" all refer to a condition

25 characterized by inappropriate, i.e., under or, more commonly,
over, PK catalytic activity, where the particular PK can be an
RTK, a CTK or an STK. Inappropriate catalytic activity can
arise as the result of either: (1) PK expression in cells
which normally do not express PKs, (2) increased PK expression

30 leading to unwanted cell proliferation, differentiation and/or
growth, or, (3) decreased PK expression leading to unwanted
reductions in cell proliferation, differentiation and/or
growth. Over-activity of a PK refers to either amplification
of the gene encoding a particular PK or production of a level

of PK activity which can correlate with a cell proliferation, differentiation and/or growth disorder (that is, as the level of the PK increases, the severity of one or more of the symptoms of the cellular disorder increases). Under-activity

5   is, of course, the converse, wherein the severity of one or more symptoms of a cellular disorder increase as the level of the PK activity decreases.

As used herein, the terms "prevent", "preventing" and "prevention" refer to a method for barring an organism from

10   acquiring a PK related disorder in the first place.

As used herein, the terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating a PK mediated cellular disorder and/or its attendant symptoms. With regard particularly to cancer, these terms simply mean

15   that the life expectancy of an individual affected with a cancer will be increased or that one or more of the symptoms of the disease will be reduced.

The term "organism" refers to any living entity comprised of at least one cell. A living organism can be as simple as,

20   for example, a single eukariotic cell or as complex as a mammal, including a human being.

The term "therapeutically effective amount" as used herein refers to that amount of the compound being administered which will relieve to some extent one or more of

25   the symptoms of the disorder being treated. In reference to the treatment of cancer, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis, (3) inhibiting

30   to some extent (that is, slowing to some extent, preferably stopping) tumor growth, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer.

It is an aspect of this invention that the above-referenced protein kinase related disorder is selected from the group consisting of a receptor protein tyrosine kinase related disorder, a cellular tyrosine kinase disorder and a

5   serine-threonine kinase related disorder.

In yet another aspect of this invention, the above referenced protein kinase related disorder is selected from the group consisting of an EGFR related disorder, a PDGFR related disorder, an IGFR related disorder and a flk related

10   disorder.

The above referenced protein kinase related disorder is a cancer selected from the group consisting of squamous cell carcinoma, sarcomas such as Kaposi's sarcoma, astrocytoma, glioblastoma, lung cancer, bladder cancer, colorectal cancer,

15   gastrointestinal cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, small-cell lung cancer and glioma in a further aspect of this invention.

The above referenced protein kinase related disorder is selected from the group consisting of diabetes, an autoimmune

20   disorder, a hyperproliferation disorder, von Hippel-Lindau disease, restenosis, fibrosis, psoriasis, osteoarthritis, rheumatoid arthritis, an inflammatory disorder and angiogenesis in yet another aspect of this invention.

Additional disorders which may be treated or prevented

25   using the compounds of this invention are immunological disorders such as autoimmune disease (AIDS) and cardiovasular disorders such as atherosclerosis.

It is as aspect of this invention that a chemical compound that modulates the catalytic activity of a protein

30   kinase may be identified by contacting cells expressing said protein kinase with a compound, salt or prodrug of the present invention and then monitoring said cells for an effect.

By "monitoring" is meant observing or detecting the effect of contacting a compound with a cell expressing a

particular PK. The observed or detected effect can be a change
in cell phenotype, in the catalytic activity of a PK or a
change in the interaction of a PK with a natural binding
partner. Techniques for observing or detecting such effects
5 are well-known in the art.

The above-referenced effect is selected from a change or
an absence of change in a cell phenotype, a change or absence
of change in the catalytic activity of said protein kinase or
a change or absence of change in the interaction of said
10 protein kinase with a natural binding partner in a final
aspect of this invention.

"Cell phenotype" refers to the outward appearance of a
cell or tissue or the biological function of the cell or
tissue. Examples, without limitation, of a cell phenotype are
15 cell size, cell growth, cell proliferation, cell
differentiation, cell survival, apoptosis, and nutrient uptake
and use. Such phenotypic characteristics are measurable by
techniques well-known in the art.

A "natural binding partner" refers to a polypeptide that
20 binds to a particular PK in a cell. Natural binding partners
can play a role in propagating a signal in a PK-mediated
signal transduction process. A change in the interaction of
the natural binding partner with the PK can manifest itself as
an increased or decreased concentration of the PK/natural
25 binding partner complex and, as a result, in an observable
change in the ability of the PK to mediate signal
transduction.

It is also an aspect of this invention that a compound
described herein, or its salt or prodrug, might be combined
30 with other chemotherapeutic agents for the treatment of the
diseases and disorders discussed above. For instance, a
compound, salt or prodrug of this invention might be combined
with alkylating agents such as fluorouracil (5-FU) alone or in
further combination with leukovorin; or other alkylating

agents such as, without limitation, other pyrimidine analogs
such as UFT, capecitabine, gemcitabine and cytarabine, the
alkyl sulfonates, e.g., busulfan (used in the treatment of
chronic granulocytic leukemia), improsulfan and piposulfan;

5   aziridines, e.g., benzodepa, carboquone, meturedepa and
uredepa; ethyleneimines and methylmelamines, e.g.,
altretamine, triethylenemelamine, triethylenephosphoramide,
triethylenethiophosphoramide and trimethylolmelamine; and the
nitrogen mustards, e.g., chlorambucil (used in the treatment

10   of chronic lymphocytic leukemia, primary macroglobulinemia and
non-Hodgkin's lymphoma), cyclophosphamide (used in the
treatment of Hodgkin's disease, multiple myeloma,
neuroblastoma, breast cancer, ovarian cancer, lung cancer,
Wilm's tumor and rhabdomyosarcoma), estramustine, ifosfamide,

15   novembrichin, prednimustine and uracil mustard (used in the
treatment of primary thrombocytosis, non-Hodgkin's lymphoma,
Hodgkin's disease and ovarian cancer); and triazines, e.g.,
dacarbazine (used in the treatment of soft tissue sarcoma).

Likewise a compound, salt or prodrug of this invention

20   might be expected to have a beneficial effect in combination
with other antimetabolite chemotherapeutic agents such as,
without limitation, folic acid analogs, e.g. methotrexate
(used in the treatment of acute lymphocytic leukemia,
choriocarcinoma, mycosis fungiodes breast cancer, head and

25   neck cancer and osteogenic sarcoma) and pteropterin; and the
purine analogs such as mercaptopurine and thioguanine which
find use in the treatment of acute granulocytic, acute
lymphocytic and chronic granulocytic leukemias.

A compound, salt or prodrug of this invention might also

30   be expected to prove efficacious in combination with natural
product based chemotherapeutic agents such as, without
limitation, the vinca alkaloids, e.g., vinblastin (used in the
treatment of breast and testicular cancer), vincristine and
vindesine; the epipodophylotoxins, e.g., etoposide and

teniposide, both of which are useful in the treatment of testicular cancer and Kaposi's sarcoma; the antibiotic chemotherapeutic agents, e.g., daunorubicin, doxorubicin, epirubicin, mitomycin (used to treat stomach, cervix, colon,

5   breast, bladder and pancreatic cancer), dactinomycin, temozolomide, plicamycin, bleomycin (used in the treatment of skin, esophagus and genitourinary tract cancer); and the enzymatic chemotherapeutic agents such as L-asparaginase.

In addition to the above, a compound, salt or prodrug of

10   this invention might be expected to have a beneficial effect used in combination with the platinum coordination complexes (cisplatin, etc.); substituted ureas such as hydroxyurea; methylhydrazine derivatives, e.g., procarbazine; adrenocortical suppressants, e.g., mitotane,

15   aminoglutethimide; and hormone and hormone antagonists such as the adrenocorticosteriods (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate); estrogens (e.g., diethylstilbesterol); antiestrogens such as tamoxifen; androgens, e.g., testosterone propionate; and aromatase

20   inhibitors (such as anastrozole.

Finally, the combination of a compound of this invention might be expected to be particularly effective in combination with mitoxantrone or paclitaxel for the treatment of solid tumor cancers or leukemias such as, without limitation, acute

25   myelogenous (non-lymphocytic) leukemia.

DETAILED DESCRIPTION OF THE INVENTION
1.   BRIEF DESCRIPTION OF THE TABLES

TABLE 1 shows the chemical structures of some exemplary compounds of this invention. The compound numbers correspond to

30   the Example numbers in the Examples section. That is, the synthesis of Compound 1 in Table 1 is described in Example 1. The compounds presented in Table 1 are exemplary only and are not to be construed as limiting the scope of this invention in any manner.

TABLE 2 shows the results of biological testing of some
exemplary compounds of this invention. The results are reported
in terms of IC_{50}, the micromolar (μM) concentration of the
compound being tested which causes a 50% change in the activity

5   of the target PKT compared to the activity of the PTK in a
control to which no test compound has been added. Specifically,
the results shown indicate the concentration of a test compound
needed to cause a 50% reduction of the activity of the target
PTK. The bioassays used are described in detail below.

10   **2.   INDICATIONS/TARGET DISEASES**

The PKs whose catalytic activity is modulated by the
compounds of this invention include protein tyrosine kinases
of which there are two types, receptor tyrosine kinases (RTKs)
and cellular tyrosine kinases (CTKs), and serine-threonine

15   kinases (STKs). RTK mediated signal transduction, is
initiated by extracellular interaction with a specific growth
factor (ligand), followed by receptor dimerization, transient
stimulation of the intrinsic protein tyrosine kinase activity
and phosphorylation. Binding sites are thereby created for

20   intracellular signal transduction molecules and lead to the
formation of complexes with a spectrum of cytoplasmic
signaling molecules that facilitate the appropriate cellular
response (e.g., cell division, metabolic effects on the
extracellular microenvironment, etc.). See, Schlessinger and

25   Ullrich, 1992, <u>Neuron</u> 9:303-391.

It has been shown that tyrosine phosphorylation sites on
growth factor receptors function as high-affinity binding
sites for SH2 (src homology) domains of signaling molecules.
Fantl et al., 1992, <u>Cell</u> 69:413-423, Songyang et al., 1994,

30   <u>Mol. Cell. Biol</u>. 14:2777-2785), Songyang et al., 1993, <u>Cell</u>
72:767-778, and Koch et al., 1991, <u>Science</u> 252:668-678.
Several intracellular substrate proteins that associate with
RTKs have been identified. They may be divided into two

principal groups: (1) substrates that have a catalytic domain, and (2) substrates which lack such domain but which serve as adapters and associate with catalytically active molecules. Songyang et al., 1993, Cell 72:767-778. The specificity of

5   the interactions between receptors and SH2 domains of their substrates is determined by the amino acid residues immediately surrounding the phosphorylated tyrosine residue. Differences in the binding affinities between SH2 domains and the amino acid sequences surrounding the phosphotyrosine

10   residues on particular receptors are consistent with the observed differences in their substrate phosphorylation profiles. Songyang et al., 1993, Cell 72:767-778. These observations suggest that the function of each RTK is determined not only by its pattern of expression and ligand

15   availability but also by the array of downstream signal transduction pathways that are activated by a particular receptor. Thus, phosphorylation provides an important regulatory step which determines the selectivity of signaling pathways recruited by specific growth factor receptors, as

20   well as differentiation factor receptors.

STKs, being primarily cytosolic, affect the internal biochemistry of the cell, often as a down-line response to a PTK event. STKs have been implicated in the signaling process which initiates DNA synthesis and subsequent mitosis leading

25   to cell proliferation.

Thus, PK signal transduction results in, among other responses, cell proliferation, differentiation, growth and metabolism. Abnormal cell proliferation may result in a wide array of disorders and diseases, including the development of

30   neoplasia such as carcinoma, sarcoma, glioblastoma and hemangioma, disorders such as leukemia, psoriasis, arteriosclerosis, arthritis and diabetic retinopathy and other disorders related to uncontrolled angiogenesis and/or vasculogenesis.

A precise understanding of the mechanism by which the compounds of this invention inhibit PKs is not required in order to practice the present invention. However, while not hereby being bound to any particular mechanism or theory, it

5   is believed that the compounds interact with the amino acids in the catalytic region of PKs. PKs typically possess a bi-lobate structure wherein ATP appears to bind in the cleft between the two lobes in a region where the amino acids are conserved among PKs. Inhibitors of PKs are believed to bind

10   by non-covalent interactions such as hydrogen bonding, van der Waals forces and ionic interactions in the same general region where the aforesaid ATP binds to the PKs. More specifically, it is thought that the 2-indolinone component of the compounds of this invention binds in the general space normally occupied

15   by the adenine ring of ATP. Specificity of a particular molecule for a particular PK may then arise as the result of additional interactions between the various substituents on the 2-indolinone core and the amino acid domains specific to particular PKs. Thus, different indolinone substituents may

20   contribute to preferential binding to particular PKs. The ability to select compounds active at different ATP (or other nucleotide) binding sites makes the compounds of this invention useful for targeting any protein with such a site. The compounds disclosed herein may thus have utility as _in_

25   _vitro_ assays for such proteins as well as exhibiting _in vivo_ therapeutic effects through interaction with such proteins.

In another aspect, the protein kinase, the catalytic activity of which is modulated by contact with a compound of this invention, is a protein tyrosine kinase, more

30   particularly, a receptor protein tyrosine kinase. Among the receptor protein tyrosine kinases whose catalytic activity can be modulated with a compound of this invention, or salt thereof, are, without limitation, EGF, HER2, HER3, HER4, IR,

IGF-1R, IRR, PDGFRα, PDGFRβ, CSFIR, C-Kit, C-fms, Flk-1R,
Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R.

      The protein tyrosine kinase whose catalytic activity is
modulated by contact with a compound of this invention, or a
5   salt or a prodrug thereof, can also be a non-receptor or
cellular protein tyrosine kinase (CTK). Thus, the catalytic
activity of CTKs such as, without limitation, Src, Frk, Btk,
Csk, Abl, ZAP70, Fes, Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck,
Blk, Hck, Fgr and Yrk, may be modulated by contact with a
10 compound or salt of this invention.

      Still another group of PKs which may have their catalytic
activity modulated by contact with a compound of this
invention are the serine-threonine protein kinases such as,
without limitation, CDK2 and Raf.

15   In another aspect, this invention relates to a method for
treating or preventing a PK related disorder by administering
a therapeutically effective amount of a compound of this
invention, or a salt or a prodrug thereof, to an organism.

      It is also an aspect of this invention that a
20 pharmaceutical composition containing a compound of this
invention or a salt or prodrug thereof is administered to an
organism for the purpose of preventing or treating a PK
related disorder.

      This invention is therefore directed to compounds that
25 modulate PK signal transduction by affecting the enzymatic
activity of RTKs, CTKs and/or STKs, thereby interfering with
the signals transduced by such proteins. More particularly,
the present invention is directed to compounds which modulate
RTK, CTK and/or STK mediated signal transduction pathways as a
30 therapeutic approach to the treatment of many kinds of solid
tumors, including but not limited to carcinomas, sarcomas
including Kaposi's sarcoma, erythroblastoma, glioblastoma,
meningioma, astrocytoma, melanoma and myoblastoma. Treatment

or prevention of non-solid tumor cancers such as leukemia are also contemplated by this invention. Indications may include, but are not limited to brain cancers, bladder cancers, ovarian cancers, gastric cancers, pancreas cancers, colon cancers,
5   blood cancers, lung cancers and bone cancers.

Further examples, without limitation, of the types of disorders related to inappropriate PK activity that the compounds described herein may be useful in preventing, treating and studying, are cell proliferative disorders,
10   fibrotic disorders and metabolic disorders.

Cell proliferative disorders, which may be prevented, treated or further studied by the present invention include cancer, blood vessel proliferative disorders and mesangial cell proliferative disorders.

15   Blood vessel proliferative disorders refer to disorders related to abnormal vasculogenesis (blood vessel formation) and angiogenesis (spreading of blood vessels). While vasculogenesis and angiogenesis play important roles in a variety of normal physiological processes such as embryonic
20   development, corpus luteum formation, wound healing and organ regeneration, they also play a pivotal role in cancer development where they result in the formation of new capillaries needed to keep a tumor alive. Other examples of blood vessel proliferation disorders include arthritis, where
25   new capillary blood vessels invade the joint and destroy cartilage, and ocular diseases, like diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness.

Two structurally related RTKs have been identified to
30   bind VEGF with high affinity: the fms-like tyrosine 1 (fit-1) receptor (Shibuya et al., 1990, Oncogene,5:519-524; De Vries et al., 1992, Science, 255:989-991) and the KDR/FLK-1 receptor, also known as VEGF-R2. Vascular endothelial growth factor (VEGF) has been reported to be an endothelial cell

specific mitogen with in vitro endothelial cell growth
promoting activity. Ferrara & Henzel, 1989, Biochein. Biophys.
Res. Comm., 161:851-858; Vaisman et al., 1990, J. Biol. Chem.,
265:19461-19566. Information set forth in U.S. application

5   Ser. Nos. 08/193,829, 08/038,596 and 07/975,750, strongly
suggest that VEGF is not only responsible for endothelial cell
proliferation, but also is the prime regulator of normal and
pathological angiogenesis. See generally, Klagsburn & Soker,
1993, Current Biology, 3(10)699-702; Houck, et al., 1992, J.

10   Biol. Chem., 267:26031-26037.

        Normal vasculogenesis and angiogenesis play important
roles in a variety of physiological processes such as
embryonic development, wound healing, organ regeneration and
female reproductive processes such as follicle development in

15   the corpus luteum during ovulation and placental growth after
pregnancy. Folkman & Shing, 1992, J. Biological Chem.,
267(16):10931-34. Uncontrolled vasculogenesis and/or
angiogenesis has been associated with diseases such as
diabetes as well as with malignant solid tumors that rely on

20   vascularization for growth. Klagsburn & Soker, 1993, Current
Biology, 3(10):699-702; Folkham, 1991, J. Natl. Cancer Inst.,
82:4-6; Weidner, et al., 1991, New Engl. J. Med., 324:1-5.

        The surmised role of VEGF in endothelial cell
proliferation and migration during angiogenesis and

25   vasculogenesis indicates an important role for the KDR/FLK-1
receptor in these processes. Diseases such as diabetes
mellitus (Folkman, 198, in XIth Congress of Thrombosis and
Haemostasis (Verstraeta, et al., eds.), pp. 583-596, Leuven
University Press, Leuven) and arthritis, as well as malignant

30   tumor growth may result from uncontrolled angiogenesis. See
e.g., Folkman, 1971, N. Engl. J. Med., 285:1182-1186. The
receptors to which VEGF specifically binds are an important
and powerful therapeutic target for the regulation and
modulation of vasculogenesis and/or angiogenesis and a variety

of severe diseases which involve abnormal cellular growth
caused by such processes. Plowman, et al., 1994, DN&P,
7(6):334-339. More particularly, the KDR/FLK-1 receptor's
highly specific role in neovascularization make it a choice
5   target for therapeutic approaches to the treatment of cancer
and other diseases which involve the uncontrolled formation of
blood vessels.

Thus, one aspect of the present invention relates to
compounds capable of regulating and/or modulating tyrosine
10   kinase signal transduction including KDR/FLK-1 receptor signal
transduction in order to inhibit or promote angiogenesis
and/or vasculogenesis, that is, compounds that inhibit,
prevent, or interfere with the signal transduced by KDR/FLK-1
when activated by ligands such as VEGF. Although it is
15   believed that the compounds of the present invention act on a
receptor or other component along the tyrosine kinase signal
transduction pathway, they may also act directly on the tumor
cells that result from uncontrolled angiogenesis.

Although the nomenclature of the human and murine
20   counterparts of the generic "flk-I" receptor differ, they are,
in many respects, interchangeable. The murine receptor, Flk-1,
and its human counterpart, KDR, share a sequence homology of
93.4% within the intracellular domain. Likewise, murine FLK-I
binds human VEGF with the same affinity as mouse VEGF, and
25   accordingly, is activated by the ligand derived from either
species. Millauer et al., 1993, Cell, 72:835-846; Quinn et
al., 1993, Proc. Natl. Acad. Sci. USA, 90:7533-7537. FLK-1
also associates with and subsequently tyrosine phosphorylates
human RTK substrates (e.g., PLC-γ or p85) when co-expressed in
30   293 cells (human embryonal kidney fibroblasts).

Models which rely upon the FLK-1 receptor therefore are
directly applicable to understanding the KDR receptor. For
example, use of the murine FLK-1 receptor in methods which

identify compounds that regulate the murine signal
transduction pathway are directly applicable to the
identification of compounds which may be used to regulate the
human signal transduction pathway, that is, which regulate

5   activity related to the KDR receptor. Thus, chemical compounds
identified as inhibitors of KDR/FLK-1 in vitro, can be
confirmed in suitable in vivo models. Both in vivo mouse and
rat animal models have been demonstrated to be of excellent
value for the examination of the clinical potential of agents

10 acting on the KDR/FLK-1 induced signal transduction pathway.

Thus, in one aspect, this invention is directed to
compounds that regulate, modulate and/or inhibit
vasculogenesis and/or angiogenesis by affecting the enzymatic
activity of the KDR/FLK-1 receptor and interfering with the

15 signal transduced by KDR/FLK-1. In another aspect, the present
invention is directed to compounds which regulate, modulate
and/or inhibit the KDR/FLK-1 mediated signal transduction
pathway as a therapeutic approach to the treatment of many
kinds of solid tumors including, but not limited to,

20 glioblastoma, melanoma and Kaposi's sarcoma, and ovarian,
lung, mammary, prostate, pancreatic, colon and epidermoid
carcinoma. In addition, data suggest the administration of
compounds which inhibit the KDR/Flk-1 mediated signal
transduction pathway may also be used in the treatment of

25 hemangioma, restenois and diabetic retinopathy.

A further aspect of this invention relates to the
inhibition of vasculogenesis and angiogenesis by other
receptor-mediated pathways, including the pathway comprising
the flt-1 receptor.

30 Receptor tyrosine kinase mediated signal transduction is
initiated by extracellular interaction with a specific growth
factor (ligand), followed by receptor dimerization, transient
stimulation of the intrinsic protein tyrosine kinase activity
and autophosphorylation. Binding sites are thereby created for

intracellular signal transduction molecules which leads to the formation of complexes with a spectrum of cytoplasmic signalling molecules that facilitate the appropriate cellular response, e.g., cell division and metabolic effects to the extracellular microenvironment. See, Schlessinger and Ullrich, 1992, _Neuron_, 9:1-20.

The close homology of the intracellular regions of KDR/FLK-1 with that of the PDGF-β receptor (50.3% homology) and/or the related flt-1 receptor indicates the induction of overlapping signal transduction pathways. For example, for the PDGF-β receptor, members of the src family (Twamley et al., 1993, _Proc. Natl. Acad. Sci. USA_, 90:7696-7700), phosphatidylinositol-3'-kinase (Hu et al., 1992, _Mol. Cell. Biol._, 12:981-990), phospholipase cγ (Kashishian & Cooper, 1993, _Mol. Cell. Biol._, 4:49-51), ras-GTPase-activating protein, (Kashishian et al., 1992, _EMBO J._, 11:1373-1382), PTP-ID/syp (Kazlauskas et al., 1993, _Proc. Natl. Acad. Sci. USA_, 10 90:6939-6943), Grb2 (Arvidsson et al., 1994, _Mol. Cell. Biol._, 14:6715-6726), and the adapter molecules Shc and Nck (Nishimura et al., 1993, _Mol. Cell. Biol._, 13:6889-6896), have been shown to bind to regions involving different autophosphorylation sites. See generally, Claesson-Welsh, 1994, _Prog. Growth Factor Res._, 5:37-54. Thus, it is likely that signal transduction pathways activated by KDR/FLK-1 include the ras pathway (Rozakis et al., 1992, _Nature_, 360:689-692), the PI-3'-kinase, the src-mediated and the plcγ-mediated pathways. Each of these pathways may play a critical role in the angiogenic and/or vasculogenic effect of KDR/FLK-1 in endothelial cells. Consequently, a still further aspect of this invention relates to the use of the organic compounds described herein to modulate angiogenesis and vasculogenesis as such processes are controlled by these pathways.

Conversely, disorders related to the shrinkage, contraction or closing of blood vessels, such as restenosis, are also implicated and may be treated or prevented by the methods of this invention.

5   Fibrotic disorders refer to the abnormal formation of extracellular matrices. Examples of fibrotic disorders include hepatic cirrhosis and mesangial cell proliferative disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the

10   formation of a hepatic scar. An increased extracellular matrix resulting in a hepatic scar can also be caused by a viral infection such as hepatitis. Lipocytes appear to play a major role in hepatic cirrhosis. Other fibrotic disorders implicated include atherosclerosis.

15   Mesangial cell proliferative disorders refer to disorders brought about by abnormal proliferation of mesangial cells. Mesangial proliferative disorders include various human renal diseases such as glomerulonephritis, diabetic nephropathy and malignant nephrosclerosis as well as such disorders as

20   thrombotic microangiopathy syndromes, transplant rejection, and glomerulopathies. The RTK PDGFR has been implicated in the maintenance of mesangial cell proliferation. Floege et al., 1993, <u>Kidney International</u> <u>43</u>:47S-54S.

Many cancers are cell proliferative disorders and, as

25   noted previously, PKs have been associated with cell proliferative disorders. Thus, it is not surprising that PKs such as, for example, members of the RTK family have been associated with the development of cancer. Some of these receptors, like EGFR (Tuzi et al., 1991, <u>Br. J. Cancer</u> 63:227-

30   233, Torp et al., 1992, <u>APMIS</u> 100:713-719) HER2/neu (Slamon et al., 1989, <u>Science</u> 244:707-712) and PDGF-R (Kumabe et al., 1992, <u>Oncogene</u>, 7:627-633) are over-expressed in many tumors and/or persistently activated by autocrine loops. In fact, in the most common and severe cancers these receptor over-

expressions (Akbasak and Suner-Akbasak et al., 1992, <u>J.</u>
<u>Neurol. Sci</u>., 111:119-133, Dickson et al., 1992, <u>Cancer</u>
<u>Treatment Res</u>. 61:249-273, Korc et al., 1992, <u>J. Clin. Invest</u>.
90:1352-1360) and autocrine loops (Lee and Donoghue, 1992, <u>J.</u>

5   <u>Cell. Biol</u>., 118:1057-1070, Korc et al., <u>supra</u>, Akbasak and
Suner-Akbasak et al., <u>supra</u>) have been demonstrated. For
example, EGFR has been associated with squamous cell
carcinoma, astrocytoma, glioblastoma, head and neck cancer,
lung cancer and bladder cancer. HER2 has been associated with

10   breast, ovarian, gastric, lung, pancreas and bladder cancer.
PDGFR has been associated with glioblastoma and melanoma as
well as lung, ovarian and prostate cancer. The RTK c-met has
also been associated with malignant tumor formation. For
example, c-met has been associated with, among other cancers,

15   colorectal, thyroid, pancreatic, gastric and hepatocellular
carcinomas and lymphomas. Additionally c-met has been linked
to leukemia. Over-expression of the c-met gene has also been
detected in patients with Hodgkins disease and Burkitts
disease.

20   IGF-IR, in addition to being implicated in nutritional
support and in type-II diabetes, has also been associated with
several types of cancers. For example, IGF-I has been implicated
as an autocrine growth stimulator for several tumor types, e.g.
human breast cancer carcinoma cells (Arteaga et al., 1989, <u>J.</u>

25   <u>Clin. Invest</u>. 84:1418-1423) and small lung tumor cells (Macauley
et al., 1990, <u>Cancer Res</u>., 50:2511-2517). In addition, IGF-I,
while integrally involved in the normal growth and
differentiation of the nervous system, also appears to be an
autocrine stimulator of human gliomas. Sandberg-Nordqvist et

30   al., 1993, <u>Cancer Res</u>. 53:2475-2478. The importance of IGF-IR
and its ligands in cell proliferation is further supported by the
fact that many cell types in culture (fibroblasts, epithelial
cells, smooth muscle cells, T-lymphocytes, myeloid cells,
chondrocytes and osteoblasts (the stem cells of the bone marrow))

are stimulated to grow by IGF-I. Goldring and Goldring, 1991, Eukaryotic Gene Expression,1:301-326. In a series of recent publications, Baserga suggests that IGF-IR plays a central role in the mechanism of transformation and, as such, could be a

5   preferred target for therapeutic interventions for a broad spectrum of human malignancies. Baserga, 1995, Cancer Res., 55:249-252, Baserga, 1994, Cell 79:927-930, Coppola et al., 1994, Mol. Cell. Biol., 14:4588-4595.

STKs have been implicated in many types of cancer

10   including, notably, breast cancer (Cance, et al., Int. J. Cancer, 54:571-77 (1993)).

The association between abnormal PK activity and disease is not restricted to cancer. For example, RTKs have been associated with diseases such as psoriasis, diabetes mellitus,

15   endometriosis, angiogenesis, atheromatous plaque development, Alzheimer's disease, von Hippel-Lindau disease, epidermal hyperproliferation, neurodegenerative diseases, age-related macular degeneration and hemangiomas. For example, EGFR has been indicated in corneal and dermal wound healing. Defects

20   in Insulin-R and IGF-1R are indicated in type-II diabetes mellitus. A more complete correlation between specific RTKs and their therapeutic indications is set forth in Plowman et al., 1994, DN&P 7:334-339.

As noted previously, not only RTKs but CTKs including, but

25   not limited to, src, abl, fps, yes, fyn, lyn, lck, blk, hck, fgr and yrk (reviewed by Bolen et al., 1992, FASEB J., 6:3403-3409) are involved in the proliferative and metabolic signal transduction pathway and thus could be expected, and have been shown, to be involved in many PTK-mediated disorders to which the

30   present invention is directed. For example, mutated src (v-src) has been shown to be an oncoprotein (pp60^{v-src}) in chicken. Moreover, its cellular homolog, the proto-oncogene pp60^{c-src} transmits oncogenic signals of many receptors. Over-expression of EGFR or HER2/neu in tumors leads to the constitutive

activation of pp60$^{c\text{-}src}$, which is characteristic of malignant cells but absent in normal cells. On the other hand, mice deficient in the expression of c-src exhibit an osteopetrotic phenotype, indicating a key participation of c-src in osteoclast function

5   and a possible involvement in related disorders.

Similarly, Zap70 has been implicated in T-cell signaling which may relate to autoimmune disorders.

STKs have been associated with inflamation, autoimmune disease, immunoresponses, and hyperproliferation disorders such as

10 restenosis, fibrosis, psoriasis, osteoarthritis and rheumatoid arthritis.

PKs have also been implicated in embryo implantation. Thus, the compounds of this invention may provide an effective method of preventing such embryo implantation and thereby be useful as

15 birth control agents.

Finally, both RTKs and CTKs are currently suspected as being involved in hyperimmune disorders.

A method for identifying a chemical compound that modulates the catalytic activity of one or more of the above

20 discussed protein kinases is another aspect of this invention.

The method involves contacting cells expressing the desired protein kinase with a compound of this invention (or its salt or prodrug) and monitoring the cells for any effect that the compound has on them. The effect may be any observable,

25 either to the naked eye or through the use of instrumentation, change or absence of change in a cell phenotype. The change or absence of change in the cell phenotype monitored may be, for example, without limitation, a change or absence of change in the catalytic activity of the protein kinase in the cells

30 or a change or absence of change in the interaction of the protein kinase with a natural binding partner.

Examples of the effect of a number of exemplary compounds of this invention on several PTKs are shown in Table 2. The

compounds and data presented are not to be construed as limiting the scope of this invention in any manner whatsoever.

3.   PHARMACEUTICAL COMPOSITIONS AND USE

A compound of the present invention, a prodrug thereof or a physiologically acceptable salt of either the compound or its prodrug, can be administered as such to a human patient or can be administered in pharmaceutical compositions in which the foregoing materials are mixed with suitable carriers or excipient(s). Techniques for formulation and administration of drugs may be found in "Remington's Pharmacological Sciences," Mack Publishing Co., Easton, PA, latest edition.

Routes of Administration.

As used herein, "administer" or "administration" refers to the delivery of a compound, salt or prodrug of the present invention or of a pharmaceutical composition containing a compound, salt or prodrug of this invention to an organism for the purpose of prevention or treatment of a PK-related disorder.

Suitable routes of administration may include, without limitation, oral, rectal, transmucosal or intestinal administration or intramuscular, subcutaneous, intramedullary, intrathecal, direct intraventricular, intravenous, intravitreal, intraperitoneal, intranasal, or intraocular injections. The preferred routes of administration ary oral and parenteral.

Alternatively, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a solid tumor, often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with tumor-specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

Composition/Formulation.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-

5   making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers

10 comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the compounds of the invention may be

15 formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are

20 generally known in the art.

For oral administration, the compounds can be formulated by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as

25 tablets, pills, lozenges, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient. Pharmaceutical preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules,

30 after adding other suitable auxiliaries if desired, to obtain tablets or dragee cores. Useful excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol, cellulose preparations such as, for example, maize starch, wheat starch, rice starch and

potato starch and other materials such as gelatin, gum
tragacanth, methyl cellulose, hydroxypropylmethyl- cellulose,
sodium carboxymethylcellulose, and/or polyvinyl- pyrrolidone
(PVP). If desired, disintegrating agents may be added, such

5   as cross-linked polyvinyl pyrrolidone, agar, or alginic acid.
A salt such as sodium alginate may also be used.

        Dragee cores are provided with suitable coatings. For
this purpose, concentrated sugar solutions may be used which
may optionally contain gum arabic, talc, polyvinyl

10   pyrrolidone, carbopol gel, polyethylene glycol, and/or
titanium dioxide, lacquer solutions, and suitable organic
solvents or solvent mixtures. Dyestuffs or pigments may be
added to the tablets or dragee coatings for identification or
to characterize different combinations of active compound

15   doses.

        Pharmaceutical compositions which can be used orally
include push-fit capsules made of gelatin, as well as soft,
sealed capsules made of gelatin and a plasticizer, such as
glycerol or sorbitol. The push-fit capsules can contain the

20   active ingredients in admixture with a filler such as lactose,
a binder such as starch, and/or a lubricant such as talc or
magnesium stearate and, optionally, stabilizers. In soft
capsules, the active compounds may be dissolved or suspended
in suitable liquids, such as fatty oils, liquid paraffin, or

25   liquid polyethylene glycols. Stabilizers may be added in
these formulations, also.

        For administration by inhalation, the compounds for use
according to the present invention are conveniently delivered
in the form of an aerosol spray using a pressurized pack or a

30   nebulizer and a suitable propellant, e.g., without limitation,
dichlorodifluoromethane, trichlorofluoromethane,
dichlorotetra- fluoroethane or carbon dioxide. In the case of
a pressurized aerosol, the dosage unit may be controlled by
providing a valve to deliver a metered amount. Capsules and

cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may also be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating materials such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of a water soluble form, such as, without limitation, a salt, of the active compound. Additionally, suspensions of the active compounds may be prepared in a lipophilic vehicle. Suitable lipophilic vehicles include fatty oils such as sesame oil, synthetic fatty acid esters such as ethyl oleate and triglycerides, or materials such as liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers and/or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

In addition to the fomulations described previously, the compounds may also be formulated as depot preparations. Such

long acting formulations may be administered by implantation
(for example, subcutaneously or intramuscularly) or by
intramuscular injection. A compound of this invention may be
formulated for this route of administration with suitable

5   polymeric or hydrophobic materials (for instance, in an
emulsion with a pharamcologically acceptable oil), with ion
exchange resins, or as a sparingly soluble derivative such as,
without limitation, a sparingly soluble salt.

A non-limiting example of a pharmaceutical carrier for

10 the hydrophobic compounds of the invention is a cosolvent
system comprising benzyl alcohol, a nonpolar surfactant, a
water-miscible organic polymer and an aqueous phase such as
the VPD co-solvent system. VPD is a solution of 3% w/v benzyl
alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80™,

15 and 65% w/v polyethylene glycol 300, made up to volume in
absolute ethanol. The VPD co-solvent system (VPD:D5W)
consists of VPD diluted 1:1 with a 5% dextrose in water
solution. This co-solvent system dissolves hydrophobic
compounds well, and itself produces low toxicity upon systemic

20 administration. Naturally, the proportions of such a co-
solvent system may be varied considerably without destroying
its solubility and toxicity characteristics. Furthermore, the
identity of the co-solvent components may be varied: for
example, other low-toxicity nonpolar surfactants may be used

25 instead of Polysorbate 80™, the fraction size of polyethylene
glycol may be varied, other biocompatible polymers may replace
polyethylene glycol, e.g., polyvinyl pyrrolidone, and other
sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic

30 pharmaceutical compounds may be employed. Liposomes and
emulsions are well known examples of delivery vehicles or
carriers for hydrophobic drugs. In addtion, certain organic

solvents such as dimethylsulfoxide also may be employed, although often at the cost of greater toxicity.

Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of

5   solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on

10   the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions herein also may comprise suitable solid or gel phase carriers or excipients. Examples

15   of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Many of the PK modulating compounds of the invention may be

20   provided as physiologically acceptable salts wherein the claimed compound may form the negatively or the positively charged species. Examples of salts in which the compound forms the positively charged moiety include, without limitation, quaternary ammonium (defined elsewhere herein), salts such as the

25   hydrochloride, sulfate, carbonate, lactate, tartrate, maleate, succinate wherein the nitrogen atom of the quaternary ammonium group is a nitrogen of the selected compound of this invention which has reacted with the appropriate acid. Salts in which a compound of this invention forms the negatively charged species

30   include, without limitation, the sodium, potassium, calcium and magnesium salts formed by the reaction of a carboxylic acid group in the compound with an appropriate base (e.g. sodium hydroxide (NaOH), potassium hydroxide (KOH), Calcium hydroxide (Ca(OH)$_2$), etc.).

Dosage.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an amount sufficient to achieve the intended purpose, i.e., the modulation of PK activity or the treatment or prevention of a PK-related disorder.

More specifically, a therapeutically effective amount means an amount of compound effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from cell culture assays. Then, the dosage can be formulated for use in animal models so as to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the PK activity). Such information can then be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC_{50} and the LD_{50} (both of which are discussed elsewhere herein) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g.,

Fingl, et al., 1975, in "The Pharmacological Basis of
Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually
to provide plasma levels of the active species which are
5   sufficient to maintain the kinase modulating effects. These
plasma levels are referred to as minimal effective
concentrations (MECs). The MEC will vary for each compound
but can be estimated from in vitro data, e.g., the
concentration necessary to achieve 50-90% inhibition of a
10   kinase may be ascertained using the assays described herein.
Dosages necessary to achieve the MEC will depend on individual
characteristics and route of administration. HPLC assays or
bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value.
15   Compounds should be administered using a regimen that
maintains plasma levels above the MEC for 10-90% of the time,
preferably between 30-90% and most preferably between 50-90%.

In cases of local administration or selective uptake, the
effective local concentration of the drug may not be related
20   to plasma concentration and other procedures known in the art
may be employed to determine the correct dosage amount and
interval.

The amount of a composition administered will, of course,
be dependent on the subject being treated, the severity of the
25   affliction, the manner of administration, the judgment of the
prescribing physician, etc.

Packaging.

The compositions may, if desired, be presented in a pack
or dispenser device, such as an FDA approved kit, which may
30   contain one or more unit dosage forms containing the active
ingredient. The pack may for example comprise metal or
plastic foil, such as a blister pack. The pack or dispenser
device may be accompanied by instructions for administration.
The pack or dispenser may also be accompanied by a notice

associated with the container in a form prescribed by a
governmental agency regulating the manufacture, use or sale of
pharmaceuticals, which notice is reflective of approval by the
agency of the form of the compositions or of human or

5   veterinary administration. Such notice, for example, may be
of the labeling approved by the U.S. Food and Drug
Administration for prescription drugs or of an approved
product insert. Compositions comprising a compound of the
invention formulated in a compatible pharmaceutical carrier

10 may also be prepared, placed in an appropriate container, and
labeled for treatment of an indicated condition. Suitable
conditions indicated on the label may include treatment of a
tumor, inhibition of angiogenesis, treatment of fibrosis,
diabetes, and the like.

15

4.   SYNTHESIS

The compounds of this invention, as well as the precursor
oxindoles and aldehydes, may be readily synthesized using

20 techniques well known in the chemical arts. It will be
appreciated by those skilled in the art that other synthetic
pathways for forming the compounds of the invention are
available and that the following is offered by way of example
and not limitation.

25   **General synthetic procedures.**

The following general methodologies may be used to
prepare intermediates to and compounds of this invention.

Preparation of substituted oxindoles

5-Amino-2-oxindole

30   5-Nitro-2-oxindole (6.3 g) was hydrogenated in methanol
over 10% palladium on carbon to give 3.0 g (60% yield) of the
title compound as a white solid.

5-Bromo-2-oxindole

2-Oxindole (1.3 g) in 20 mL acetonitrile was cooled to -

10 °C and 2.0 g N-bromosuccinimide was slowly added with
stirring. The reaction was stirred for 1 hour at -10 °C and 2
hours at 0 °C. The precipitate was collected, washed with water
and dried to give 1.9 g (90 % yield) of the title compound.

5   **4-Methyl-2-oxindole**

Diethyl oxalate (30 mL) in 20 mL of dry ether was added
with stirring to 19 g of potassium ethoxide suspended in 50 mL
of dry ether. The mixture was cooled in an ice bath and 20 mL
of 3-nitro-o-xylene in 20 mL of dry ether was slowly added.

10   The thick dark red mixture was heated to reflux for 0.5 hr,
concentrated to a dark red solid, and treated with 10% sodium
hydroxide until almost all of the solid dissolved. The dark
red mixture was treated with 30% hydrogen peroxide until the
red color changed to yellow. The mixture was treated

15   alternately with 10% sodium hydroxide and 30% hydrogen
peroxide until the dark red color was no longer present. The
solid was filtered off and the filtrate acidified with 6N
hydrochloric acid. The resulting precipitate was collected by
vacuum filtration, washed with water, and dried under vacuum

20   to give 9.8 g (45% yield) of 2-methyl-6-nitrophenylacetic acid
as an off-white solid. The solid was hydrogenated in methanol
over 10 % palladium on carbon to give 9.04 g of the title
compound as a white solid.

7-Bromo-5-chloro-2-oxindole

25   5-Chloro-2-oxindole (16.8 g) and 19.6 g of N-
bromosuccinimide were suspended in 140 mL of acetonitrile and
refluxed for 3 hours. Thin layer chromatography (silica, ethyl
acetate) at 2 hours of reflux showed 5-chloro-2-oxindole or N-
bromosuccinimide (Rf -0.8), product (Rf 0.85) and a second

30   product (Rf 0.9) whose proportions did not change after
another hour of reflux. The mixture was cooled to 10 °C, the
precipitate was collected by vacuum filtration, washed with 25
mL of ethanol and sucked dry for 20 minutes in the funnel to

give 14.1 g of wet product (56 % yield). The solid was
suspended in 200 mL of denatured ethanol and slurry-washed by
stirring and refluxing for 10 minutes. The mixture was cooled
in an ice bath to 10 °C. The solid product was collected by
5   vacuum filtration, washed with 25 mL of ethanol and dried
under vacuum at 40 °C to give 12.7 g (51% yield) of 7-bromo-5-
chloro-2-oxindole.

5-Fluoro-2-oxindole

5-Fluoroisatin (8.2 g) was dissolved in 50 mL of
10 hydrazine hydrate and refluxed for 1.0 hr. The reaction
mixtures were then poured in ice water. The precipitate was
then filtered, washed with water and dried in a vacuum oven to
afford the title compound.

5-Nitro-2-oxindole

15   2-Oxindole (6.5 g) was dissolved in 25 mL concentrated
sulfuric acid and the mixture maintained at -10 to -15 °C while
2.1 mL of fuming nitric acid was added dropwise. After the
addition of the nitric acid the reaction mixture was stirred
at 0 °C for 0.5 hr and poured into ice-water. The precipitate
20 was collected by filtration, washed with water and
crystallized from 50% acetic acid. The crystalline product was
then filtered, washed with water and dried under vacuum to
give 6.3 g (70%) of 5-nitro-2-oxindole.

5-Aminosulfonyl-2-oxindole

25   To a 100 mL flask charged with 27 mL of chlorosulfonic
acid was added slowly 13.3 g of 2-oxindole. The reaction
temperature was maintained below 30 °C during the addition.
After the addition, the reaction mixture was stirred at room
temperature for 1.5 hr, heated to 68 °C for 1 hr, cooled, and
30 poured into water. The precipitate was washed with water and
dried in a vacuum oven to give 11.0 g of 5-chlorosulfonyl-2-
oxindole (50% yield) which was used without further
purification.

5-Chlorosulfonyl-2-oxindole (2.1 g) was added to 10 mL of ammonium hydroxide in 10 mL of ethanol and stirred at room temperature overnight. The mixture was concentrated and the solid collected by vacuum filtration to give 0.4 g (20% yield)

5   of the title compound as an off-white solid.

5-Methylaminosulfonyl-2-oxindole

A suspension of 3.38 g of 5-chlorosulfonyl-2-oxindole in 10 mL 2M methylamine in tetrahydrofuran was stirred at room temperature for 4 hours during which time a white solid

10   formed. The precipitate was collected by vacuum filtration, washed twice with 5 mL of water and dried under vacuum at 40 °C overnight to give 3.0 g (88 % yield) of 5-methylaminosulfonyl-2-oxindole.

5-(4-Trifluoromethylphenylaminosulfonyl)-2-oxindole

15   A suspension of 2.1 g of 5-chlorosulfonyl-2-oxindole, 1.6 g of 4-trifluoromethylaniline and 1.4 g of pyridine in 20 mL of dichloromethane was stirred at room temperature for 4 hours. The precipitate which formed was collected by vacuum filtration, washed twice with 5 mL of water and dried under

20   vacuum at 40 °C overnight to give 2.4 g of crude product containing some impurities by thin layer chromatography. The crude product was chromatographed on silica gel eluting with ethyl acetate:hexane (1:2) to give 1.2 g (37 % yield) of 5-(4-trifluoromethylphenyl-aminosulfonyl)-2-oxindole.

25   **5-(Morpholinosulfonyl)-2-oxindole**

A suspension of 2.3 g of 5-chlorosulfonyl-2-oxindole and 2.2 g of morpholine in 50 mL of dichloromethane was stirred at room temperature for 3 hours. The white precipitate was collected by vacuum filtration, washed with ethyl acetate and

30   hexane and dried under vacuum at 40 °C overnight to give 2.1 g (74 % yield) of 5-(morpholinosulfonyl)-2-oxindole.

6-Trifluoromethyl-2-oxindole

Dimethylsulfoxide (330 mL) was added to 7.9 g of sodium hydride followed by dropwise addition of 43.6 g diethyloxalate. The mixture was heated to 100 °C for 1.0 hour and cooled to room temperature. 2-Nitro-4-

5   trifluoromethyltoluene (31.3 g) was added, the reaction stirred for 30 minutes at room temperature and then heated to 100 °C for 1 hour. The reaction was cooled and poured into a mixture of saturated aqueous ammonium chloride, ethyl acetate and hexane. The organic layer was washed with saturated

10   ammonium chloride, water and brine, dried, and concentrated to give dimethyl 2-(2-nitro-4-trifluoromethylphenyl)malonate.

The diester was dissolved in a mixture of 6.4 g of lithium chloride and 2.7 mL of water in 100 mL of dimethylsulfoxide and heated to 100 °C for 3 hours. The

15   reaction was cooled and poured into a mixture of ethyl acetate and brine. The organic phase was washed with brine, dried with sodium sulfate, concentrated and chromatographed on silica gel (10 % ethyl acetate in hexane). The fractions containing product were evaporated to give 25.7 g of methyl 2-nitro-4-

20   trifluoromethylphenylacetate.

Methyl 2-nitro-4-trifluoromethylphenylacetate (26 mg) was hydrogenated over 10 % palladium on carbon and then heated at 100 °C for 3 hours. The catalyst was removed by filtration and the solvent evaporated to give the title compound.

25   **4-Carboxy-2-oxindole**

A solution of trimethylsilyldiazomethane in hexane (2M) was added dropwise to a solution of 2.01 g of 2-chloro-3-carboxy-nitrobenzene in 20 mL methanol at room temperature until no further gas evolution occurred. The excess trimethylsilyldiazo-

30   methane was quenched with acetic acid. The reaction mixture was dried by rotary pump and the residue was further dried in a vacuum oven overnight. The product (2-chloro-3-methoxycarbonyl-nitrobenzene) was pure enough for the following reaction.

Dimethyl malonate (6.0 mL) was added to an ice-cold suspension of 2.1 g of sodium hydride in 15 mL of DMSO. The reaction mixture was then stirred at 100 °C for 1.0 h and then cooled to room temperature. 2-Chloro-3-methoxycarbonyl-

5 nitrobenzene (2.15 g) was added to the above mixture in one portion and the mixture was heated to 100 °C for 1.5 h. The reaction mixture was then cooled to room temperature and poured into ice water, acidified to pH 5, and extracted with ethyl acetate. The organic layer was washed with brine, dried

10 over anhydrous sodium sulfate and concentrated to give 3.0 g of the dimethyl 2-methoxycarbonyl-6-nitrophenylmalonate.

Dimethyl 2-methoxycarbonyl-6-nitrophenylmalonate (3.0 g) was refluxed in 50 mL of 6 N hydrochloric acid overnight. The mixture was concentrated to dryness and refluxed for 2 hours

15 with 1.1 g of tin(II) chloride in 20 mL of ethanol. The mixture was filtered through Celite, concentrated and chromatographed on silica gel (ethyl acetate:hexane:acetic acid) to give 0.65 g (37% yield) of 4-carboxy-2-oxindole as a white solid.

20 **5-Carboxyethyl-2-oxindole**

5-Cyanoethyl-2-oxindole (4.02 g) in 10 mL of water containing 25 mL of concentrated hydrochloric acid was refluxed for 4 hours. The mixture was cooled, water added and the resulting solid collected by vacuum filtration, washed

25 with water and dried to give 1.9 g (44% yield) of the title compound as a yellow solid.

5-(Morpholin-4-ethyl)-2-oxindole

5-Chloroethyl-2-oxindole (2.3 g), 1.2 mL of morpholine and 1.2 mL of diisopropylethylamine were heated overnight at

30 100 °C in 10 mL of dimethylsulfoxide. The mixture ws cooled, poured into water and extacted with ethyl acetate. The organic layer was washed with brine, dried and evaporated. The residue

was chromatographed on silica gel (5 % methanol in chloroform)
to give 0.9 g (31%) of the title compound as a white solid.

5-(4-Methoxycarbonylbenzamido)-2-oxindole

A mixture of 82.0 mg 5-amino-2-oxindole and 131.0 mg 4-

5 methoxycarbonylbenzoyl chloride in pyridine was stirred at
room temperature for 3 hr and poured into ice water. The
precipitate was filtered, washed with water and dried in a
vacuum oven to give 138.0 mg of 5-(4-
methoxycarbonylbenzamido)-2-oxindole (81% yield).

10 **5-Methoxy-2-oxindole**

Chloral hydrate (9.6 g) was dissolved in 200 mL of water
containing 83 g of sodium sulfate. The solution was warmed to
60 °C, a solution of 11.4 g of hydroxylamine hydrochloride in
50 mL of water was added and the mixture was held at 60 °C. In

15 a separate flask, 6.4 g of 4-anisidine and 4.3 mL of
concentrated hydrochloric acid in 80 mL of water was warmed to
80 °C. The first solution was added to the second and the
mixture refluxed for 2 minutes after which it was cooled
slowly to room temperature and then cooled in an ice bath. The

20 tan precipitate was collected by vacuum filtration, washed
with water and dried under vacuum to give 8.6 g (85% yield)
of N-(2-hydroximino-acetyl)anisidine.

Concentrated sulfuric acid (45 mL) containing 5 mL of
water was warmed to 60 °C and 8.6 g of N-(2-

25 hydroximinoacetyl)anisidine was added in one portion. The
stirred mixture was heated to 93 °C for 10 minutes and then
allowed to cool to room temperature. The mixture was poured
into 500 g of ice and extracted 3 times with ethyl acetate.
The combined extracts were dried over anhydrous sodium sulfate

30 and concentrated to give 5.1 g (65% yield) of 5-methoxyisatin
as a dark red solid. 5-Methoxyisatin (5.0 g) and 30 mL of
hydrazine hydrate were heated to reflux for 15 minutes. The
reaction mixture was cooled to room temperature and 50 mL of

water was added. The mixture was extracted 3 times with 25 mL
of ethyl acetate each time, the organic layers combined, dried
over anhydrous sodium sulfate and concentrated to give a
yellow solid. The solid was stirred in ethyl acetate and 1.1 g

5   of insoluble material was removed by vacuum filtration and
saved. This material proved to be 2-hydrazinocarbonylmethyl-4-
anisidine. The filtrate was concentrated and chromatographed
on silica gel eluting with ethyl acetate:hexane (1:1) to give
0.7 g of 5-methoxy-2-oxindole as a yellow solid. The 1. 1 g of

10 2-hydrazino- carbonylmethyl-4-anisidine was refluxed for 1
hour in 20 mL of 1N sodium hydroxide. The mixture was cooled,
acidified to pH 2 with concentrated hydrochloric acid and
extracted 3 times with 25 mL of ethyl acetate each time. The
organic extracts were combined, washed with brine, dried over

15 anhydrous sodium sulfate and concentrated to give 0.8 g of 5-
methoxy-2-oxindole as a yellow solid. The combined yield was
1.5 g or 33%.

7-Azaoxindole

3,3-Dibromo-7-azaoxindole (2.9 g) was dissolved in a

20 mixture of 20 mL of acetic acid and 30 mL of acetonitrile. To
the solution was added 6.5 g of zinc dust. The mixture was
stirred for 2 hrs at room temperature. The solid was filtered
from the mixture and the solvent evaporated. The residue was
slurried with ethyl acetate. The ethyl acetate solution

25 containing insoluble solid was passed through a short column
of silica gel. The collected ethyl acetate solution was
evaporated and the residue dried under vacuum to give 1.8 g
(yield 91%) of 7-azaoxindole acetic acid salt.

5-Dimethylaminosulfonyl-2-oxindole

30   A suspension of 2.3 g 5-chlorosulfonyl-2-oxindole in 10
mL 2M dimethylamine in methanol was stirred at room
temperature for 4 hours at which time a white solid formed.
The precipitate was collected by vacuum filtration, washed
with 5 mL of 1N sodium hydroxide and 5 mL of 1N hydrochloric

acid and dried under vacuum at 40 °C overnight to give 1.9 g
(79% yield) of 5-dimethylamino- sulfonyl-2-oxindole.

6-Phenyl-2-oxindole

5   Dimethyl malonate (10 mL) in 25 mL of dimethylsulfoxide
was added dropwise to 3.5 g sodium hydride suspended in 25 mL
dimethylsulfoxide and the mixture heated at 100 °C for 10
minutes. The mixture was cooled to room temperature and 4.7 g
of 4-fluoro-3-nitrobiphenyl in 25 mL dimethylsulfoxide was
added. The mixture was heated at 100 °C for 2 hours, cooled and

10   quenched with 300 mL of saturated ammonium chloride solution.
The mixture was extracted three times with ethyl acetate and
the combined organic layers washed with water and brine and
evaporated to give, as a yellow oil, crude dimethyl-3-
nitrobiphenyl- 4-malonate.

15   Crude dimethyl-3-nitrobiphenyl-4-malonate was refluxed in
30 mL of 6 N hydrochloric acid for 24 hours. The precipitate
was collected by filtration, washed with water and dried to
give 4.5 g of 3-nitrobiphenyl-4-acetic acid as a cream colored
solid.

20   Iron powder (2.6 g) was added all at once to 4.5 g of 3-
nitrobiphenyl-4-acetic acid in 40 mL of acetic acid. The
mixture was refluxed for 2 hours, concentrated to dryness and
taken up in ethyl acetate. The solids were removed by
filtration and the filtrate washed twice with 1N hydrochloric

25   acid and brine and dried over anhydrous sodium sulfate. The
filtrate was concentrated to give 3.4 g (93% yield) of 6-
phenyl-2-oxindole as a light brown solid.

6-(3-Methoxyphenyl)-2-oxindole

     Tetrakis(triphenylphosphine)palladium (0.8 g) was added

30   to a mixture of 5 g 3-methoxyphenylboronic acid, 5 g 5-bromo-
2-fluoro- nitrobenzene and 11 mL of 2 M sodium carbonate
solution in 100 mL of toluene. The mixture was refluxed for 2
hours, diluted with water and extracted with ethyl acetate.

The ethyl acetate was washed with saturated sodium bicarbonate and brine and then dried and concentrated to give an oily solid. The solid was chromatographed on silica gel (ethyl acetate:hexane (1:6)) to give 4.3 g (77% yield) of 4-fluoro-

5   3'-methoxy-3- nitrobiphenyl.

Dimethyl malonate (9.7 mL) was added dropwise to 2.0 g sodium hydride suspended in 50 mL dimethylsulfoxide. The mixture was heated to 100 °C for 35 minutes and cooled to room temperature. 4-Fluoro-2'-methoxy-3-nitrobiphenyl (4.2 g) in 50

10   mL dimethylsulfoxide was added and the mixture was heated at 100 °C for 1 hour. The reaction mixture was cooled and quenched with 300 mL of saturated ammonium chloride solution and extracted twice with ethyl acetate. The extracts were combined, washed with brine, dried over anhydrous sodium

15   sulfate and concentrated to give crude dimethyl 3'-methoxy-3-nitrobiphenyl-4-malonate as a pale yellow solid.

Crude dimethyl 3'-methoxy-3-nitrobiphenyl-4-malonate was heated at 110 °C in 45 mL 6N hydrochloric acid for 4 days and then cooled. The precipitate was collected by filtration,

20   washed with water and hexane, and dried to give 5.3 g of 3'-methoxy-2- nitrobiphenyl-4-acetic acid as a light tan solid.

3'-Methoxy-3-nitrobiphenyl-4-acetic acid (5.2 g) was dissolved in methanol and hydrogenated over 0.8 g of 10% palladium on carbon for 3 hours at room temperature. The

25   catalyst was removed by filtration, washed with methanol and the filtrates combined and concentrated to give a brown solid. The solid was chromatographed on silica gel in ethyl acetate:hexane:acetic acid (33:66:1) to give 3.0 g of 6-(3-methoxypheny)-2-oxindole as a pink solid.

30   **5-Acetyl-2-oxindole**

2-Oxindole (3 g) was suspended in 1,2-dichloroethane and 3.2 mL acetyl chloride were slowly added. The resulting suspension was heated to 50 °C for 5 hours, cooled, and poured

into water. The resulting precipitate was collected by vacuum
filtration, washed copiously with water and dried under vacuum
to give 2.9 g (73% yield) of the title compound as a brown
solid.

5   **5-Cyanoethyl-2-oxindole**

Potassium cyanide (2.0 g) was added to 15 mL of dimethyl-
sulfoxide and heated to 90 °C. 5-Chloroethyl-2-oxindole (3.0
g) dissolved in 5 mL dimethyl sulfoxide was added slowly with
stirring, and the reaction heated to 150 °C for 2 hours. The

10   mixture was cooled, poured into ice water and the precipitate
collected by vacuum filtration, washed with water, dried and
then chromatographed on silica gel (5% methanol in chloroform)
to give 1.2 g (42% yield) of the title compound.

6-(Morpholin-4-yl)-2-oxindole

15   6-Amino-2-oxindole (2.2 g), 4.0 g 2, 2'-dibromoethyl
ether and 7.9 g sodium carbonate were refluxed in 20 ml
ethanol overnight, concentrated and diluted with 50 ml of
water. The mixture was extracted three times with 50 ml of
ethyl acetate and the organic extracts combined, washed with

20   20 ml of brine, dried over anhydrous sodium sulfate and
concentrated to dryness. The solid was chromatographed on a
column of silica gel (ethyl acetate:hexane (1:1) containing
0.7% acetic acid) to give 1.2 g (37% yield) of the title
compound as a beige solid.

25   **Methylation of phenols**

An appropriate phenol (1 equivalent) is stirred overnight
at room temperature with an equal volume of dimethylformamide
containing 1.3 equivalents of methyl iodide and 1.3
equivalents of potassium carbonate. The mixture is diluted

30   with water and extracted with ethyl acetate. The ethyl acetate
layer is washed with water and brine. The organic layer is
separated, dried over anhydrous sodium sulfate and evaporated
to dryness to give the anisole derivative.

Formylation of anisoles

The anisole (1 equivalent), dimethylformamide (1-3
equivalents) and phosphorus oxychloride (1-3 equivalents) are
heated to 100° C for 1-3 hours. The reaction mixture is cooled
5   to room temperature and dichloromethane is added. The mixture
is cooled in ice and water is then added, followed by 4-12
equivalents of concentrated sodium hydroxide until a pH of 9-
10 is reached. The organic layer is separated and washed with
water and then with brine, dried over anhydrous sodium sulfate
10 and evaporated to give the crude aldehyde. The crude
aledehyde is dissolved in boiling hexane containing activated
carbon and the hexane solution is decanted and filtered hot
through a layer of silica gel. The filtrate is evaporated to
dryness to give the benzaldhyde derivative.

15   ### Formylation of pyrroles

$POCl_3$ (1.1 equiv.) is added dropwise to dimethylformamide
(3 equiv.) in dichloromethane at -10° C followed by the
appropriate pyrrole. After stirring for two hours, the
reaction mixture is diluted with H_2O and basified to pH 11 with
20 10 N KOH. The precipitate which forms is collected by
filtration, washed with H_2O and dried in a vacuum oven to give
the desired aldehyde.

Saponification of pyrrolecarboxylic acid esters

A mixture of a pyrrolecarboxylic acid ester and KOH (2 –
25 4 equiv.) in EtOH is refluxed until reaction completion is
indicated by thin layer chromatography (TLC). The cooled
reaction mixtrue is acidified to pH 3 with 1 N HCl. The
precipitate which forms is collected by filtration, washed
with H_2O and dried in a vacuum oven to give the desired
30 pyrrolecarboxylic acid.

Amidation of pyrrolecarboxylic acids

To a stirred solution of a pyrrolecarboxylic acid
dissolved in dimethylformamide(0.3M) is added 1-ethyl-3-(3-

dimethylaminopropyl)carboiimide (1.2 equiv.), 1-
hydroxybenzotriazole (1.2 equiv.), and triethylamine (2
equiv.). The appropriate amine is added (1 equiv.) and the
reaction stirred until completion is indicated by TLC. The
5   reaction is diluted with saturated $NaHCO_3$ and brine (with extra
salt), dried over anhydrous $MgSO_4$ and concentrated to afford
the desired amide.

Preparation of pyrrole and indole carbaldehydes

3-(2-Formyl-5-methyl-1*H*-pyrrol-3-yl)propionic acid

10   4-(2-Carboxyethyl)-3-ethoxycarbonyl-2-methylpyrrole
(Bulter, A.R., and George, S.D. (1993) Tetrahedron <u>49</u>: 7017-
7026) was hydrolyzed using potassium hydroxide (KOH) in
ethanol (EtOH) to give 4-(2-carboxyethyl)-2-methyl-1*H*-pyrrole-
3-carboxylic acid (64%).

15   ^{1}HNMR (DMSO-d_6) δ 11.63 (s, 1H, COO*H*), 10.81 (s, 1H, N*H*),
6.36 (s, 1H, C*H*), 2.78, 2.45 (2 x t, 4H, CH$_2$CH$_2$), 2.34(s, 3H,
C*H*$_3$).

            MS *m/z* 225 [M+2].

            4-(2-Carboxyethyl)-2-methyl-1*H*-pyrrole-3-carboxylic acid
20 was decarboxylated by heating for one hour at 200° C to give 3-
(5-methyl-1*H*-pyrrol-3-yl)propionic acid.

            ^{1}HNMR (DMSO-d_6) δ 10.07 (s, 1H, N*H*), 9.60 (br s, 1H,
COO*H*), 6.30 (s, 1H, C*H*), 5.56 (s, 1H, C*H*), 2.51, 2.36 (2 x t,
4H, CH$_2$CH$_2$), 2.10(s, 3H, C*H*$_3$).

25   MS *m/z* 154 [M+1].

            3-(5-Methyl-1*H*-pyrrol-3-yl)propionic acid was formylated
using phosphorus oxycloride (POCl$_3$) and N,N-dimethylformamide
(DMF) to give 3-(2-formyl-5-methyl-1*H*-pyrrol-3-yl)propionic
acid.

30   ^{1}HNMR (300 MHz, DMSO-d_6) δ 12.09 (br s, 1H, COO*H*), 11.54
(br s, 1H, N*H*), 9.44 (s, 1H, CHO), 5.86 (d, *J* = 1.8 Hz, 1H),
2.87 (t, *J* = 7.5 Hz, 2H, C*H*$_2$), 2.47 (t, *J* = 7.5 Hz, 2H, C*H*$_2$),
2.16 (s, 3H, C*H*$_3$).

MS-EI m/z 181 [M$^+$].

4-(2-Carboxyethyl)-5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester

To a suspension of 10 g (38.9 mmol) ethyl 2,4-dimethyl-

5   5-(ethoxycarbonyl)-3-pyrrolepropionate (commercially available) in a mixture of water (250 mL) and methanol (MeOH)(50 mL) was added 3.0 g of KOH in 50 mL of water. The mixture was stirred at 70° C for 4 hr. The reaction mixture was cooled and the methanol was stripped from the mixture

10   under vacuum. The remaining solution was filtered and the filtrate was then acidified with 6N hydrochloric acid to pH 3. The precipitate which formed was collected by filtration, washed with a 2:1 mixture of water and ethanol and dried under vacuum to give 7.5 g of 4-(2-carboxy-ethyl)-3,5-

15   dimethyl-1H-pyrrole-2-carboxylic acid ethyl ester as a white solid.

^{1}HNMR (360 MHz, DMSO-d_6) δ 12.03 (br s, 1H, COOH), 11.02 (br s, 1H, NH), 4.16 (q, J = 7.2 Hz, 2H, OCH_2CH$_3$), 2.53 (t, J = 7.8 Hz, 2H, CH_2CH$_2$), 2.25 (t, J = 7.8 Hz, 2H, CH$_2$CH_2), 2.16 (s,

20   3H, CH$_3$), 2.11 (s, 3H, CH$_3$), 1.25 (t, J = 7.2 Hz, 3H, OCH$_2$CH_3).

MS-EI m/z 239 [M$^+$].

To a mixture of 7 g 4-(2-carboxyethyl)-3,5-dimethyl-1H-pyrrole-2-carboxylic acid ethyl ester in 35 mL each of tetrahydrofuran (THF), acetic acid (AcOH) and water at -10° C

25   was added 70 g of ceric ammonium nitrate in portion over 20 minutes, the reaction temperature being maintained at about 5° C. The resulting mixture was them cooled to 0° C, stirred for another 2 hr and then diluted with 250 mL of brine. The mixture was then extracted with 2 x 300 mL 10% MeOH in

30   dichloromethane (DCM). The organic extracts were combined and dried over anhydrous magnesium sulfate (MgSO$_4$), filtered and concentrated. The residue was purified on silica gel column with hexanes(hex)/ethyl acetate (EtOAc)/AcOH (6:4:0.05)

followed by crystallization in EtOAc to give 1.5 g of 4-(2-carboxyethyl)-5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester as a white solid.

5   ^{1}HNMR (360 MHz, DMSO-d_6) δ 12.36 (br s, 1H, COOH), 12.01 (br s, 1H, NH), 9.77 (s, 1H, CHO), 4.27 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 2.89 (t, J = 7.6 Hz, 2H, CH_2CH$_2$), 2.37 (t, J = 7.6 Hz, 2H, CH$_2$CH_2), 2.21 (s, 3H, CH_3), 1.30 (t, J = 7.1 Hz, 3H, OCH$_2$CH_3).

MS-EI m/z 253 [M$^+$].

10   **4-(3-Dimethylaminopropyl)-5-formyl-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester**

To a suspension of 4-(2-carboxyethyl)-3-ethoxycarbonyl-2-methylpyrrole (2 g, 8.88 mmol) (Bulter, A.R., and George, S.D. (1993) Tetrahedron 49: 7017-7026) in 18 mL of DMF was added

15   1.73 g (10.65 mmol) 1,1'-carbonyldiimidazole (CDI) followed by the dropwise addition of 8.9 mL (17.76 mmol) of 2M dimethylamine in THF. After stirring for 2 hr, the reaction was diluted with water (200 mL) and cooled. The precipitate which formed was collected by filtration, washed with water

20   and dried to give 1.0 g of the product as a white crystalline solid. The filtrate was extracted with EtOAc, the organic layer was washed with brine, dried and concentrated to obtain an additional 0.9 g of the product for a total of 1.9 g of 4-(2-dimethylcarbamoyl-ethyl)-2-methyl-1H-pyrrole-3-carboxylic

25   acid ethyl ester (85%).

^{1}HNMR (360 MHz, DMSO-d_6) δ 10.89 (br s, 1H, NH), 6.40 (d, J = 2.5 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 2.92 (s, 3H, NCH_3), 2.79 (s, 3H, NCH_3), 2.75 (m, 2H, CH_2CH$_2$), 2.45 (m, 2H, CH$_2$CH_2), 2.35 (s, 3H, CH_3), 1.23 (t, J = 7.1 Hz, 3H,

30   OCH$_2$CH_3).

MS-EI m/z 252 [M$^+$].

To a heterogeneous mixture of 4-(2-dimethylcarbamoyl-ethyl)-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester (1.01

g, 4 mmol) in THF (9 mL) was added dropwise 8 mL of borane-tetrahydrofuran complex (1M in THF). The mixture was refluxed overnight. Then 9.0 mL of MeOH was added slowly to the reaction and refluxing was continued for another 2 hr. The

5 reaction mixture was cooled, quenched with 1N HCl and extracted with EtOAc. The aqueous layer was basified with aqueous KOH and extracted with EtOAc. The EtOAc extract was washed with brine, dried and concentrated to give 616 mg (65%) of 4-(3-dimethylaminopropyl)-2-methyl-1H-pyrrole-3-carboxylic

10 acid ethyl ester as a faint orange oil.

^{1}HNMR (360 MHz, DMSO-d_6) δ 10.84 (br s, 1H, NH), 6.36 (d, J = 2.5 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 2.52 (m, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.17 (m, 2H, CH_2), 2.08 (s, 6H, N(CH_3)$_2$), 1.57 (m, 2H, CH$_2$CH_2 CH$_2$), 1.23 (t, J = 7.1 Hz, 3H,

15 OCH$_2$CH_3).

MS-EI m/z 238 [M$^+$].

4-(3-Dimethylaminopropyl)-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester (600 mg, 2.5 mmol) was formylated using POCl$_3$ and DMF to give 645 mg (96%) of 4-(3-dimethylamino-

20 propyl)-5-formyl-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester.

^{1}HNMR (360 MHz, DMSO-d_6) δ 12.15 (br s, 1H, NH), 9.59 (s, 1H, CHO), 4.19 (q, J = 7.2 Hz, 2H, OCH_2CH$_3$), 2.93 (m, 2H, CH_2), 2.41 (s, 3H, CH_3), 2.18 (m, 2H, CH_2), 2.09 (s, 6H, N(CH_3)$_2$),

25 1.63 (m, 2H, CH$_2$CH_2 CH$_2$), 1.27 (t, J = 7.2 Hz, 3H, OCH$_2$CH_3).

MS-EI m/z 266 [M$^+$].

3-(3-Dimethylaminopropyl)-1H-indole-2-carbaldehyde

Ethyl indole-2-carboxylate (10 g, 52.8 mmol) was formylated using POCl$_3$ (1.3 equiv.) and DMF (1.3 equiv.) as

30 above to give 3-formyl-1H-indole-2-carboxylic acid ethyl ester as a white solid.

^{1}HNMR (360 MHz, DMSO-d_6) δ 12.0 (br s, 1H, NH), 10.6 (s, 1H, CHO), 8.24 (dd, J = 0.7 & 8.0 Hz, 1H), 7.57 (d, 1H), 7.38

(m, 1H), 7.26 (m, 1H), 4.44 (q, J = 7.12 Hz, 2H, OCH_2CH_3), 1.39 (t, J = 7.12 Hz, 3H, OCH_2CH_3).

MS-EI m/z 217 [M⁺].

Butyllithium (1.1 equiv.) was added to a suspension of
5   (2-dimethylaminoethyl)triphenylphosphonium bromide in THF (0.2 M) at 0° C. After stirring for 30 minutes, lithium diethylamine (1.1 equiv.) was added dropwise followed by the cold suspension of 3-formyl-1H-indole-2-carboxylic acid ethyl ester (3.96 g, 18.2 mmol) in THF. The resulting orange suspension
10   was stirred for 18 hours. The reaction was then quenched with saturated ammonium chloride and extracted with 10% MeOH in DCM. The organic layer was washed with saturated sodium bicarbonate, dried and concentrated to give 3-(3-dimethylaminopropenyl)-1H-indole-2-carboxylic acid ethyl ester
15   as a brownish-red waxy solid.

^{1}HNMR (360 MHz, DMSO-d_6) δ 11.8 (br s, 1H, NH), 7.93 (d, 1H), 7.49 (d, 1H), 7.41 (d, 1H), 7.3 (t, 1H), 7.14 (t, 1H), 6.33-6.42 (m, 1H), 4.36 (q, 2H, OCH_2CH_3), 3.38 (d, 2H, CH_2), 2.44 (s, 6H, 2xCH_3), 1.36 (t, 3H, OCH_2CH_3).

20   MS-EI m/z 272 [M⁺].

3-(3-Dimethylaminopropenyl)-1H-indole-2-carboxylic acid ethyl ester was hydrogenated using 10% palladium over carbon to reduce the double bond followed by a lithium aluminum hydride (LAH) reduction of the ester group to an alcohol
25   resulting in the formation of [3-(3-dimethylaminopropyl)-1H-indol-2-yl]methanol as an orange oil.

Manganese dioxide (10 equiv.) was added to a solution of [3-(3-dimethylaminopropyl)-1H-indol-2-yl]methanol (2.2 g) in DCM. The mixture was stirred at room temperature for 18 hr.
30   Insoluble materials were removed by filtrationand the filtrate was concentrated. The residue was column chromatographed to give 3-(3-dimethylaminopropyl)-1H-indole-2-carbaldehyde as a cream-colored solid.

¹HNMR (300 MHz, DMSO-d_6) δ 11.58 (br s, 1H, NH), 10.0 (s, 1H, CHO), 7.72 (d, 1H), 7.39 (d, 1H), 7.29 (t, 1H), 7.07 (t, 1H), 3.08 (t, 2H, CH_2), 2.20 (t, 2H), 2.12 (s, 6H, 2xCH_3), 1.73 (t, 2H, CH_2).

MS-EI m/z 230 [M⁺].

Preparation of aminotetrahydoindolecarbaldehydes

A mixture of 5-aminolevulinic acid hydrochloride (1 equiv.), 1,3-cyclohexanedione (1 equiv.) and sodium acetate (2 equiv.) in water (1M) is stirred at 110° C for 4 - 12 hr and then cooled. The precipitate which forms is collected by vacuum filtration, washed with 30% ethanol in water and dried under vacuum to give the amido-keto-tetrahydroindole in 50-70% yield.

Lithium aluminum hydride (LAH, 4 equiv.) is added dropwise to a suspension of the appropriate amido-keto-tetrahydroindole (1 equiv.) in THF (0.5 M). The mixture is refluxed overnight. The mixture is then cooled and water is added carefully until no more gas is generated, then a few drops of 15% NaOH/water is added. The mixture is then stirred at room temperature for 0.5 hr and filtered to remove insoluble materials. The filtrate is concentrated to give the amino-tetrahydroindole.

Examples of syntheses of aminotetrahydroindolecarbaldehydes

The following syntheses of aminotetrahydroindole-carbaldehydes are shown by way of example only and are not to be construed as limiting the scope of this invention in any manner whatsoever.

3-[3-(4-methylpiperazin-1-yl)propyl]-4,5,6,7-tetrahydro-1H-indole-2-carbaldehyde

Step 1: A mixture of 5-aminolevulinic acid hydrochloride (1.68 g, 10 mmol), 1,3-cyclohexanedione (1.12 g, 10 mmol) and sodium acetate (1.64 g, 20 mmol) in water (10 mL) was stirred at 110° C for 4 hr and then cooled. The precipitate which

formed was collected by vacuum filtration, washed with 30% of ethanol in water and dried under vacuum to give 1.7 g (82%) of 3-(4-oxo-4,5,6,7-tetrahydro-1*H*-indol-3-yl)-propionic acid.

^{1}HNMR (360 MHz, DMSO-d6) δ 11.91 (br s, 1H, COO*H*), 10.99
5   (br s, 1H, N*H*), 6.45 (d, *J* = 1.4 Hz, 1H), 2.76 (t, 2H, C*H$_2$*), 2.69 (t, 2H, C*H$_2$*), 2.44 (t, 2H, C*H$_2$*), 2.26 (t, 2H, C*H$_2$*), 1.96 (m, 2H, C*H$_2$*).

MS-EI *m/z* 207 [M$^+$].

Step 2: To a suspension of 10 g of the product of step 1
10   (48 mmol) in dichloromethane(60 mL) was added 9.3 g (57.6 mmol) of 1,1'-carbonyldiimidazole. The mixture was stirred at room temperature for 2 hours and then 5.3 mL (48 mmol) 1-methylpiperazine and 8.4 mL (48 mmol) N,N-diisopropylethyl-amine was added. The dark red reaction mixture was then
15   stirred at room temperature overnight. The reaction was then poured into water, the organic layer separated and washed with brine, dried and concentrated to give 8 g (57%) of 3-[3-(4-methylpiperazin-1-yl)-3-oxo-propyl]-1,5,6,7-tetrahydroindol-4-one.

20   ^{1}HNMR (360 MHz, DMSO-d6) δ 10.97 (br s, 1H, N*H*), 6.47 (d, *J* = 2.0 Hz, 1H), 3.43 (m, 4H, 2xC*H$_2$*), 2.67-2.75 (m, 4H, 2xC*H$_2$*), 2.51 (m, 2H, C*H$_2$*), 2.27 (m, 2H, C*H$_2$*), 2.20 (m, 4H, 2xC*H$_2$*), 2.15 (s, 3H, C*H$_3$*), 1.97 (m, 2H, C*H$_2$*).

MS-EI *m/z* 289 [M$^+$].

25   **Step 3:** LAH (2.6 g, 68 mmol) was added dropwise to a suspension of 3-[3-(4-methylpiperazin-1-yl)-3-oxo-propyl]-1,5,6,7-tetrahydroindol-4-one (5g, 17 mmol) in THF (300 mL). The mixture was then refluxed overnight. The mixture was then cooled and 2.6 mL each of water followed by a few drops of 15%
30   NaOH was added. The reaction was stirred at room temperature for 30 min and then filtered to remove insolubles. The filtrate was concentrated to give 4.5 g (100%) of 3-[3-(4-methylpiperazin-1-yl)propyl]-4,5,6,7-tetrahydro-1*H*-indole.

¹HNMR (360 MHz, DMSO-d6) δ 9.79 (br s, 1H, N*H*), 6.22 (d, *J* = 2.0 Hz, 1H), 2.44 (m, 2H, C*H₂*), 2.21-2.3 (m, 14H, 7xC*H₂*), 2.12 (s, 3H, C*H₃*), 1.65 (m, 4H, 2xC*H₂*), 1.53 (m, 2H, C*H₂*).

MS-EI *m/z* 261 [M⁺].

5   **Step 4:** POCl₃ (1.8 mL, 18.9 mmol) was added dropwise to N,N-dimethylformamide(DMF, 3.8 mL, 51.6 mmol) at -5° C. The mixture was then allowed to come to room temperature and then stirred for 30 minutes after which it was again cooled to -5°C. A solution of 3-[3-(4-methylpiperazin-1-yl)-propyl]-4,5,6,7-

10   tetrahydro-1*H*-indole (4.5 g, 17.2 mmol) in DMF (9 mL) was added dropwise. The mixture was again allowed to come to room temperature and then stirred at that temperature overnight. The reaction was then quenched with ice, followed by 10 N NaOH to adjust the pH to 10-11. After stirring at room temperature

15   for 1 hr, the reaction was extracted with ethyl acetate (EtOAc), the organic layer separated, washed with brine, dried and concentrated to give 3.1 g (62%) of 3-[3-(4-methylpiperazin-1-yl)-propyl]-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde.

20   ¹HNMR (360 MHz, DMSO-d6) δ 11.24 (br, s, 1H, N*H*), 9.42 (s, 1H, C*H*O), 2.60 (t, 2H, C*H₂*), 2.51 (m, 2H, C*H₂*), 2.35 (m, 2H, C*H₂*), 2.28 (m, 8H, 4xC*H₂*), 2.21 (m, 2H, C*H₂*), 2.12 (s, 3H, C*H₃*), 1.57-1.68 (m, 6H, 3xC*H₂*).

MS-EI *m/z* 289 [M⁺].

25   **3-(3-dimethylaminopropyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde**

The procedure was the same as that employed in Example A except that the amine in Step 2 was dimethylamine (2.0M solution in tetrahydrofuran).

30   **Step 2:** <u>*N,N*-Dimethyl-3-(4-oxo-4,5,6,7-tetrahydro-1*H*-indol-3-yl)-propionamide</u>:

¹HNMR (300 MHz, DMSO-d6) δ 11.0 (br s, 1H, N*H*), 6.48 (d, *J* = 1.6 Hz, 1H), 2.95 (s, 3H, C*H₃*), 2.79 (s, 3H, C*H₃*), 2.71 (m,

4H, 2xCH_2), 2.47 (m, 2H, CH_2), 2.27 (t, 2H, CH_2), 1.96 (m, 2H, CH_2).

MS-EI m/z 234 [M⁺].

Step 3: <u>Dimethyl-[3-(4,5,6,7-tetrahydro-1H-indol-3-yl)propyl]-amine</u>:

¹HNMR (300 MHz, DMSO-d6) δ 9.83 (br s, 1H, NH), 6.22 (d, J = 2.3 Hz, 1H), 2.43 (m, 2H, CH_2), 2.28 (m, 2H, CH_2), 2.14-2.25 (m, 4H, CH_2), 2.08 (s, 6H, N(CH_3)$_2$), 1.64 (m, 4H, 2xCH_2), 1.53 (m, 2H, CH_2).

MS-EI m/z 206 [M⁺].

Step 4: <u>3-(3-Dimethylaminopropyl)-4,5,6,7-tetrahydro-1H-indole-2-carbaldehyde</u>:

¹HNMR (300 MHz, DMSO-d6) δ 11.29 (br, s, 1H, NH), 9.40 (s, 1H, CHO), 2.59 (t, 2H, CH_2), 2.5 (m, 2H, CH_2), 2.34 (m, 2H, CH_2), 2.16 (m, 2H, CH_2), 2.08 (s, 6H, N(CH_3)$_2$), 1.67 (m, 4H, 2xCH_2), 1.56 (m, 2H, CH_2).

MS-EI m/z 234 [M⁺].

3-(3-pyrrolidin-1-ylpropyl)-4,5,6,7-tetrahydro-1H-indole-2-carbaldehyde

The procedure employed was that same as that in Example A except that the amine used in Step 2 was pyrrolidine.

Step 2: <u>3-(3-Oxo-3-pyrrolidin-1-ylpropyl)-1,5,6,7-tetrahydroindol-4-one</u>:

¹HNMR (300 MHz, DMSO-d6) δ 11.05 (br s, 1H, NH), 6.46 (d, J = 1.5 Hz, 1H), 3.35 (m, 2H, CH_2), 2.24 (m, 2H, CH_2), 2.66-2.73 (m, 4H, 2xCH_2), 2.44 (m, 2H, CH_2), 2.26 (m, 2H, CH_2), 1.96 (m, 2H, CH_2), 1.82 (m, 2H, CH_2), 1.73 (m, 2H, CH_2).

MS-EI m/z 260 [M⁺].

Step 3: <u>3-(3-Pyrrolidin-1-ylpropyl)-4,5,6,7-tetrahydro-1H-indole</u>:

¹HNMR (300 MHz, DMSO-d6) δ 9.82 (br s, 1H, NH), 6.22 (s, 1H), 2.2-2.5 (m, 12H, 6xCH_2), 1.5-1.64(m, 10H, 5xCH_2).

MS-EI *m/z* 232 [M⁺].

Step 4: 3-(3-Pyrrolidin-1-ylpropyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde:

¹HNMR (300 MHz, DMSO-d6) δ 11.28 (br, s, 1H, N*H*), 9.38 (s, 1H, C*H*O), 2.59 (t, 2H, C*H₂*), 2.46 (m, 2H, C*H₂*), 2.3-2.44 (m, 8H, 4xC*H₂*), 1.55-1.65 (m, 10H, 5xC*H₂*).

MS-EI *m/z* 260 [M⁺].

3-(3-diethylamino-propyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde

The procedure used was the same as that in Example A except that the amine used was diethylamine.

Step 2: *N,N*-Diethyl-3-(4-oxo-4,5,6,7-tetrahydro-1*H*-indol-3-yl)-propionamide:

¹HNMR (300 MHz, DMSO-d6) δ 11.0 (br s, 1H, N*H*), 6.47 (d, *J* = 2.0 Hz, 1H), 3.24 (m, 4H, N(C*H₂*CH₃)₂), 2.69 (m, 4H, 2xC*H₂*), 2.46 (m, 2H, C*H₂*), 2.27 (m, 2H, C*H₂*), 1.96 (m, 2H, C*H₂*), 1.04 (t, *J* = 7.0 Hz, 3H, NCH₂C*H₃*), 0.98 (t, *J* = 7.0 Hz, 3H, NCH₂C*H₃*).

Step 3: Diethyl-[3-(4,5,6,7-tetrahydro-1*H*-indol-3-yl)-propyl]-amine:

¹HNMR (300 MHz, DMSO-d6) δ 9.83 (br s, 1H, N*H*), 6.22 (d, *J* = 2.0 Hz, 1H), 2.19-2.44 (m, 12H, 6xC*H₂*), 1.64 (m, 4H, 2xC*H₂*), 1.51 (m, 2H, C*H₂*), 0.90 (t, *J* = 7.0 Hz, 6H, N(CH₂C*H₃*)₂).

MS-EI *m/z* 234 [M⁺].

Step 4: 3-(3-Diethylaminopropyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde:

¹HNMR (360 MHz, DMSO-d6) δ 11.24 (br, s, 1H, N*H*), 9.42 (s, 1H, C*H*O), 2.59 (t, 2H, C*H₂*), 2.34-2.53 (m, 10H, 5xC*H₂*), 1.67 (m, 4H, 2xC*H₂*), 1.57 (m, 2H, C*H₂*), 0.91 (t, *J* = 7.0 Hz, 6H, N(CH₂C*H₃*)₂).

MS-EI *m/z* 262 [M⁺].

3-(3-Diethylaminopropyl)-6,6-dimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde

Step 1: A mixture of 5-aminolevulinic acid hydrochloride

(1.68 g, 10 mmol) , 5,5-dimethyl-1,3-cyclohexandione (1.4 g,
10 mmol) and sodium acetate (1.64 g, 20 mmol) in water (10 mL)
was stirred at 110° C for 4 hr and then cooled. The precipitate
5   which formed was collected by vacuum filtration, washed with
30% of ethanol (EtOH) in water and dried under vacuum to give
1.6 g (68%) of 3-(4-oxo-6-dimethyl-4,5,6,7-tetrahydro-1*H*-
indol-3-yl)propionic acid.

   ^{1}HNMR (360 MHz, DMSO-d6) δ 11.89 (br s, 1H, COO*H*), 10.94
10 (br s, 1H, N*H*), 6.45 (d, J = 1.4 Hz, 1H), 2.76 (t, 2H, C*H$_2$*),
   2.57 (s, 2H, C*H$_2$*), 2.44 (t, 2H, C*H$_2$*), 2.16 (s, 2H, C*H$_2$*), 0.99
   (s, 6H, 2xC*H$_3$*).

   MS-EI *m/z* 235 [M$^+$].

   Step 2: To a suspension of 1.18 g (5 mmol) of the product
15 of step 1 in dichloromethane (25 mL) was added 0.97 g (6 mmol)
   of CDI. After stirring at room temperature for 2 hr, 2.1 mL
   (20 mmol) diethylamine was added. The mixture was stirred at
   room temperature overnight. The reaction was concentrate and
   the residue was dissolved in dichloromethane, washed with
20 brine, dried and concentrated to give 1.2 g (83%) of 3-(6,6-
   dimethyl-4-oxo-4,5,6,7-tetrahydro-1*H*-indol-3-yl)-*N,N*-diethyl-
   propionamide as a white solid.

   ^{1}HNMR (360 MHz, DMSO-d6) δ 10.91 (br s, 1H, N*H*), 6.46 (s,
   1H), 3.20-3.29 (m, 4H, 2xC*H$_2$*), 2.72-2.76 (m, 2H, C*H$_2$*), 2.57 (s,
25 2H, C*H$_2$*), 2.45 (m, 2H, C*H$_2$*), 2.17 (s, 2H, C*H$_2$*), 0.96-1.06 (m,
   12H, 4xC*H$_3$*).

   MS-EI *m/z* 290 [M$^+$].

   Step 3: LAH (0.57 g, 15.1 mmol) was added dropwise to a
   suspension of 3-(6,6-dimethyl-4-oxo-4,5,6,7-tetrahydro-1*H*-
30 indol-3-yl)-*N,N*-diethylpropionamide (1.1 g, 3.8 mmol) in THF
   (80 mL). The mixture was refluxed overnight. The reaction was
   cooled and ice was added until no more gas was generated. A
   few drops of 15% NaOH in water was then added. The mixture was

stirred at room temperature for 30 minutes and then filtered to remove insolubles. The filtrate was concentrated to give 0.9 g of [3-(6,6-dimethyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propyl]-diethyl-amine as a light yellow oil.

5   ^{1}HNMR (360 MHz, DMSO-d6) δ 9.75 (br s, 1H, N*H*), 6.24 (s, 1H), 2.19-2.44 (m, 14H, 7x*CH₂*), 1.53 (m, 2H, *CH₂*), 1.40 (m, 2H, *CH₂*), 0.88-0.92 (m, 12H, 4x*CH₃*).

    MS-EI *m/z* 262 [M⁺].

    Step 4: POCl₃ (0.35 mL, 3.74 mmol) was added dropwise to 10   DMF (0.8 mL, 10.3 mmol) at -5° C. After stirring at room temperature for 30 min, the mixture was cooled to -5°C. A solution of [3-(6,6-dimethyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propyl]-diethylamine (0.9 g, 3.4 mmol) in DMF (2 mL) was then added dropwise. The mixture was stirred at room 15   temperature for 3 hours and then quenched with ice, followed by 10 N KOH to adjust pH to 10-11. After stirring at room temperature for 1 hour, the mixture was extracted with EtOAc, washed with brine, dried and concentrated to give 0.55 g 3-(3-diethylaminopropyl)-6,6-dimethyl-4,5,6,7-tetrahydro-1H-indole-20   2-carbaldehyde.

    ^{1}HNMR (360 MHz, DMSO-d6) δ 11.23 (br, s, 1H, N*H*), 9.41 (s, 1H, C*H*O), 2.61 (t, 2H, *CH₂*), 2.30-2.43 (m, 10 H, 5x *CH₂*), 1.58 (m, 2H, *CH₂*), 1.45 (t, 2H, *CH₂*), 0.93 (s, 6H, 2x*CH₃*), 0.89 (t, 6H, N(CH₂*CH₃*)₂).

25   MS-EI *m/z* 290 [M⁺].

6,6-Dimethyl-3-(3-pyrrolidin-1-ylpropyl)-4,5,6,7-tetrahydro-1H-indole-2-carbaldehyde

    The procedure employed was the same as that in Example E except that the amine in Step 2 was pyrrolidine.

30   **Step 2:** <u>6,6-Dimethyl-3-(3-oxo-3-pyrrolidin-1-ylpropyl)-1,5,6,7-tetrahydroindol-4-one</u>

    ^{1}HNMR (360 MHz, DMSO-d6) δ 10.90 (br s, 1H, N*H*), 6.46 (s, 1H), 3.34 (t, 2H, *CH₂*), 3.25 (t, 2H, *CH₂*), 2.76 (m, 2H, *CH₂*),

2.58 (s, 2H, C*H*₂), 2.43 (m, 2H, C*H*₂), 2.17 (s, 2H, C*H*₂), 1.74-
1-1.74 (m, 4H, 2xC*H*₂), 1.00 (s, 6H, 2xC*H*₃).

MS-EI *m/z* 288 [M⁺].

Step 3: <u>6,6-Dimethyl-3-(3-pyrrolidin-1-ylpropyl)-4,5,6,7-</u>
<u>tetrahydro-1*H*-indole</u>

¹HNMR (360 MHz, DMSO-d6) δ 9.75 (br s, 1H, N*H*), 6.23 (d,
1H), 2.22-2.37 (m, 12H, 6xC*H*₂), 1.55-1.66 (m, 6H, 3xC*H*₂), 1.40
(m, 2H, C*H*₂), 0.92 (s, 6H, 2xC*H*₃).

MS-EI *m/z* 260 [M⁺].

Step 4: <u>6,6-Dimethyl-3-(3-pyrrolidin-1-ylpropyl)-4,5,6,7-</u>
<u>tetrahydro-1*H*-indole-2-carbaldehyde</u>

¹HNMR (360 MHz, DMSO-d6) δ 11.25 (br, s, 1H, N*H*), 9.41 (s,
1H, C*H*O), 2.64 (t, 2H, C*H*₂), 2.31-2.38 (m, 10 H, 5x C*H*₂), 1.59-
1.67 (m, 6H, 3xC*H*₂), 1.46 (t, 2H, C*H*₂), 0.93 (s, 6H, 2xC*H*₃).

MS-EI *m/z* 288 [M⁺].

6,6-Dimethyl-3-[3-(4-methylpiperazin-1-yl)-propyl]-4,5,6,7-
tetrahydro-1*H*-indole-2-carbaldehyde

The procedure used was that same as that in Example E
except that the amine in Step 2 was 1-methylpiperazine.

Step 2: <u>6,6-Dimethyl-3-[3-(4-methylpiperazin-1-yl)-3-oxo-</u>
<u>propyl]-1,5,6,7-tetrahydroindol-4-one</u>

¹HNMR (360 MHz, DMSO-d6) δ 10.93 (br s, 1H, N*H*), 6.48 (s,
1H), 3.42 (m, 4H, 2xC*H*₂), 3.73 (m, 2H, C*H*₂), 2.57 (s, 2H, C*H*₂),
2.48 (m, 2H, C*H*₂), 2.21 (m, 4H, 2xC*H*₂), 2.17 (s, 2H, C*H*₂), 2.15
(s, 3H, C*H*₃), 1.00 (s, 6H, 2xC*H*₃).

MS-EI *m/z* 317 [M⁺].

Step 3: <u>6,6-Dimethyl-3-[3-(4-methylpiperazin-1-yl)-</u>
<u>propyl]-4,5,6,7-tetrahydro-1*H*-indole</u>

¹HNMR (360 MHz, DMSO-d6) δ 9.74 (br s, 1H, N*H*), 6.24 (s,
1H), 2.21-2.30 (m, 16H, 8xC*H*₂), 2.12 (s, 3H, C*H*₃), 1.56 (m, 2H,
C*H*₂), 1.40 (m, 2H, C*H*₂), 0.92 (s, 6H, 2xC*H*₃).

MS-EI *m/z* 289 [M⁺].

Step 4: <u>6,6-Dimethyl-3-[3-(4-methylpiperazin-1-yl)-</u>

propyl]-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde

^{1}HNMR (360 MHz, DMSO-d6) δ 11.21 (br, s, 1H, N*H*), 9.42 (s, 1H, C*H*O), 2.62 (t, 2H, C*H*$_2$), 2.18-2.37 (m, 14 H, 7x C*H*$_2$), 2.12 (s, 3H, C*H*$_3$), 1.61 (m, 2H, C*H*$_2$), 1.45 (t, 2H, C*H*$_2$), 0.93 (s, 6H, 2xC*H*$_3$).

MS-EI *m/z* 317 [M⁺].

6,6-Dimethyl-3-(3-morpholin-4-ylpropyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde

The procedure used was the same as that in Example E except that the amine used was morpholine.

Step 2: 6,6-Dimethyl-3-(3-morpholin-4-yl-3-oxopropyl)-1,5,6,7-tetrahydroindol-4-one

^{1}HNMR (360 MHz, DMSO-d6) δ 10.94 (br s, 1H, N*H*), 6.49 (s, 1H), 3.42-3.50 (m, 8H, 4xC*H*$_2$), 2.74 (m, 2H, C*H*$_2$), 2.57 (s, 2H, C*H*$_2$), 2.48 (m, 2H, C*H*$_2$), 2.17 (s, 2H, C*H*$_2$), 1.00 (s, 6H, 2xC*H*$_3$).

MS-EI *m/z* 304 [M⁺].

Step 3: 6,6-Dimethyl-3-(3-morpholin-4-ylpropyl)-4,5,6,7-tetrahydro-1*H*-indole

^{1}HNMR (360 MHz, DMSO-d6) δ 9.75 (br s, 1H, N*H*), 6.24 (s, 1H), 3.54 (m, 4H, 2xC*H*$_2$), 2.23-2.31 (m, 12H, 6xC*H*$_2$), 1.58 (m, 2H, C*H*$_2$), 1.40 (m, 2H, C*H*$_2$), 0.92 (s, 6H, 2xC*H*$_3$).

MS-EI *m/z* 276 [M⁺].

Step 4: 6,6-Dimethyl-3-(3-morpholin-4-ylpropyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde

^{1}HNMR (360 MHz, DMSO-d6) δ 11.25 (br, s, 1H, N*H*), 9.43 (s, 1H, C*H*O), 3.54 (m, 4H, 2xC*H*$_2$), 2.63 (t, 2H, C*H*$_2$), 2.20-2.37 (m, 10 H, 5xC*H*$_2$), 1.62 (m, 2H, C*H*$_2$), 1.45 (t, 2H, C*H*$_2$), 0.93 (s, 6H, 2xC*H*$_3$).

MS-EI *m/z* 304 [M⁺].

3-(3-Dimethylaminopropyl)-6,6-dimethyl-4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde

The procedure was the same as that in Example E except that the amine used was dimethylamine.

Step 2: <u>3-(6,6-Dimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indol-3-yl)-N,N-dimethylpropionamide</u>

^{1}HNMR (360 MHz, DMSO-d6) δ 10.93 (br s, 1H, NH), 6.48 (s, 1H), 2.94 (s, 3H, CH$_3$), 2.79 (s, 3H, CH$_3$), 2.72 (m, 2H, CH$_2$), 2.57 (s, 2H, CH$_2$), 2.48 (m, 2H, CH$_2$), 2.17 (s, 2H, CH$_2$), 1.00 (m, 6H, 2xCH$_3$).

MS-EI *m/z* 262 [M$^+$].

Step 3: <u>[3-(6,6-Dimethyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propyl]dimethylamine</u>

^{1}HNMR (360 MHz, DMSO-d6) δ 9.75 (br s, 1H, NH), 6.24 (s, 1H), 3.36 (m, 2H, CH$_2$), 2.07-2.3 (m, 14H, 7xCH$_2$), 1.53 (m, 2H, CH$_2$), 1.40 (m, 2H, CH$_2$), 0.92 (s, 6H, 2xCH$_3$).

MS-EI *m/z* 234 [M$^+$].

Step 4: <u>3-(3-Dimethylaminopropyl)-6,6-dimethyl-4,5,6,7-tetrahydro-1H-indole-2-carbaldehyde</u>

^{1}HNMR (360 MHz, DMSO-d6) δ 11.25 (br s, 1H, NH), 9.41 (s, 1H, CHO), 2.62 (t, 2H, CH$_2$), 2.36 (t, 2H, CH$_2$), 2.30 (s, 2H, CH$_2$), 2.21 (t, 2H, CH$_2$), 2.12 (s, 6H, 2xCH$_3$), 1.60 (m, 2H, CH$_2$), 1.46 (t, 2H, CH$_2$), 0.93 (s, 6H, 2xCH$_3$).

MS-EI *m/z* 262 [M$^+$].

<u>**Condensation of aldehydes and oxindoles containing carboxylic acid substituents**</u>

A mixture of the appropriate oxindole (1 equivalent), 1 equivalent of the appropriate aldehyde and 1 - 3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100° C until the reaction complete as indicated by thin layer chromatography. The mixture is then concentrated and the residue is triturated with dilute hydrochloric acid. The resulting precipitate is collected by vacuum filtration, washed with water and ethanol and dried to give the product.

<u>**Condensation of aldehyde and oxindole not containing carboxylic acid substituents**</u>

A mixture of the appropriate oxindole (1 equivalent), 1 equivalent of the appropriate aldehyde and 1 - 3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100° C until the reaction complete as indicated by thin

5   layer chromatography. The mixture is cooled to room temperature and the precipitate which forms is collected by vacuum filtration, washed with ethanol and dried to give the product. Where a precipitate does not form, the mixture is concentrated and the product isolated by column

10   chromatography.

Condensation of aldehyde and oxindole containing amino substituents

The aminooxindole is first protected with a BOC-group. After the condensation with the appropriate aldehyde,

15   the solid which forms is deprotected using trifluoroacetic acid to yield the desired product.

C.   **Examples - Synthesis of 3-methylidenyl-2-indolinones of this invention.**

The following syntheses of representative compounds of

20   this invention are shown by way of example only and are not to be construed as limiting the scope of this invention in any manner whatsoever.

Example 1:   3-(3,5-Diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

25   3,5-Diisopropyl-4-hydroxybenzaldehyde was methylated to give 3,5-diisopropyl-4-methoxybenzaldehyde.

^{1}HNMR (d_6-DMSO) δ: 10.1 (s, 1H, CHO), 6.9 (s, 2H, aromatic), 3.9 (s, 3H, OCH$_3$), 3.2 (m, 2H, 2xCH), 1.2 (d, 12H, 4xCH$_3$).

30   3,5-Diisopropyl-4-methoxybenzaldehyde was then condensed with 2-oxindole to give 0.25 g of 3-(3,5-Diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

¹HNMR (d₆-DMSO) δ: 10.54 (s, 1H, CONH), 7.6, 7.59, 7.49, 7.21, 6.81-6.88 (multiplets, 7H, =CH-, aromatic), 3.73 (s, 3H, OCH₃), 3.26-3.33 (m, 2H, 2x-CH), 1.2 (d, J = 7.23Hz, 12H, 4xCH₃).

MS m/z 336.

Example 2:   5-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

¹HNMR (d₆-DMSO) δ: 10.69 (s, 1H, CONH), 7.67 (s, 1H, =CH-), 7.6 (d, J = 1.85Hz, 1H, H-4), 7.51 (s, 2H, aromatic), 7.26 (dd, J = 1.85, 8.3Hz, 1H, H-6), 6.89 (d, J = 8.3Hz, 1H, H-7), 3.74 (s, 3H, OCH₃), 3.27-3.34 (m, 2H, 2x-CH), 1.22 (d, J = 6.7Hz, 12H, 4xCH₃).

Example 3:   N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide

Tin chloride dihydrate (225 g) was added to a solution of 2,4-dinitrophenylacetic acid (22.6 g) in ethanol (450 ml). The mixture was heated at 90° C for 10 hours. The reaction mixture was cooled and adjusted to pH 11 with 12M sodium hydroxide. The solids were removed by filtration and the filtrate was concentrated. The residue was treated with ethanol (300 ml). Insoluble materials were removed by filtration and washed with ethanol (5 x 60 ml). The combined ethanol solutions were evaporated and the solid obtained was dried under vacuum to give 15g of 6-amino-2-oxindole as a brown powder.

¹HNMR (360 MHz, DMSO-d6) δ: 10.03 (s, br, NH), 6.78 (d, J = 8.55Hz, 1H, H-4), 6.09-6.11 (m, 2H), 4.95 (s, br, 2H, NH2), 3.22 (s, 2H, H-3).MS (+ APCI) m/z (relative intensity, %)

MS m/z 147 ([M-1]', 100).

To a mixture of 6-amino-2-oxindole (1 g) and acetyl

chloride (0.79 g) in 10 ml of dichloromethane at 0° C was added triethylamine (1 g). The mixture was brought to room temperature and stirred for 4 hours protected from atmospheric moisture with a dry-tube. The solvent was then evaporated and

5   the residue was recrystallized from methanol to give 0.98 g (77%) of 6-acetylamino-2-oxindole as a beige solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.28 (s, br, NH), 9.83 (s, br, NH), 7.34 (d, J = 1.4Hz, 1H, H-7), 7.06 (d, J = 7.9Hz, 1H, H-4), 6.97 (dd, J = 1.4, 7.9Hz, 1H, H-5), 3.37 (s, 2H, H-3),

10   2.01 (s, 3H, CH$_3$).

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 6-acetamido-2-oxindole to give 0.3 g of N-[3-(3,5-diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide as a yellow-orange solid.

15   ^{1}HNMR (d$_6$-DMSO) δ: 10.48 (s, 1H, CONH), 9.99 (s, 1H, CONH), 7.51 (d, J = 8Hz, 1H, H-4), 7.46 (s, 2H, aromatic), 7.44 (s, 1H, =CH-), 7.41 (d, J = 2Hz, 1H, H-7), 6.91 (dd, J = 2,8Hz, 1H, H-5), 3.73 (s, 3H, OCH$_3$), 3.26-3.34 (m, 2H), 2.03 (s, 3H, CH$_3$), 1.21 (d, J = 7Hz, 12H, 4xCH$_3$).

20   MS m/z 392.

Example 4:   3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-hydroxy-1,3-dihydroindol-2-one

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 6-hydroxy-2-oxindole to give 0.3 g of 3-(3,5-Diisopropyl-4-

25   methoxybenzylidene)-6-hydroxy-1,3-dihydroindol-2-one as a. yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.34 (s, 1H, CONH), 9.79 (s, 1H, OH), 7.42 (d, J = 8Hz, 1H, H-4), 7.43 (s, 2H, aromatic), 7.33 (s, 1H, =CH-), 6.32 (d, J = 2Hz, 1H, H-7), 6.21 (dd, J = 2,8Hz,

30   1H, H-5), 3.72 (s, 3H, OCH$_3$), 3.25-3.33 (m, 2H), 1.2 (d, J = 7Hz, 12H, 4xCH$_3$).

MS m/z 351.

Example 5:   5-Acetyl-3-(3,5-diisopropyl-4-methoxybenzylidene)-

1,3-dihydroindol-2-one

2-Oxindole (3 g) was suspended in 1,2-dichloroethane and slowly treated with 3.2 ml of acetyl chloride. The resulting suspension was heated at 50° C for 5 hours, cooled, and poured
5   into water. The resulting precipitate was collected by vacuum filtration, washed copiously with water and dried under vacuum to give 2.9 g (73% yield) of 5-acetyl-2-oxindole as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.75 (s, br, NH), 7.83 (d, J
10 = 8.23Hz, 1H), 7.78 (s, 1H, H-4), 6.88 (d, J = 8.23Hz, 1H), 3.53 (s, 2H, CH2), 2.49 (s, 3H, CH3).

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5-acetyl-2-oxindole to give 0.3 g of 5-Acetyl-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a
15 yellow-orange solid.

^{1}HNMR (d_6-DMSO) δ: 10.95 (s, 1H, CONH), 8.22 (d, J = 2Hz, 1H, H-4), 7.93 (dd, J = 2,8Hz, 1H, H-6), 7.69 (s, 1H, =CH-), 7.53 (s, 2H, aromatic), 6.97 (d, J = 8Hz, 1H, H-7), 3.75 (s, 3H, OCH$_3$), 3.24-3.34 (m, 2H), 2.48 (s, 3H, CH$_3$), 1.24 (d, J =
20 7Hz, 12H, 4xCH$_3$).

MS m/z 377.

Example 6:   3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester

2-Oxindole (82.9 g) was suspended in 630 ml of acetic
25 acid in a reaction vessel equipped with mechanical stirring and the mixture cooled to 10° C in an ice water bath. Solid N-iodosuccinimide (175 g) was added in portions over 10 minutes. After the addition was complete the mixture was stirred for 1 hour at 10° C. The suspended solid which was always present
30 became very thick at this time. The solid was collected by vacuum filtration, washed with 100 ml of 50% acetic acid in water and then with 200 ml of water and subjected to vacuum filtration for 20 minutes to partially air dry it. The product

was then dried under vacuum to give 93.5 g (36%) of 5-iodo-2-oxindole containing about 5% 2-oxindole by proton NMR.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.45 (s, 1H, NH-1), 7.49 (s, 1H, H-4), 7.48 (d, J = 8.10 Hz, 1H, H-6), 6.64 (d, J = 8.10

5   Hz, 1H, H-7), and 3.46 (s, 2H, CH$_2$-3).

MS (m/z (relative intensity, %)) 258 ([M-1]$^+$, 13).

5-Iodo-2-oxindole (17 g) was refluxed with 2 g of palladium diacetate, 18.2 g of triethylamine, 150 ml of methanol, 15 ml of dimethylsulfoxide and 2.6 g of DPPP in an

10   atmosphere saturated with carbon monoxide. After 24 hours, the reaction was filtered to remove the catalyst and the filtrate was concentrated. The concentrate was chromatographed on silica gel using 30% ethyl acetate/hexane as the eluent. The fractions containing product were concentrated and allowed to

15   stand. The precipitated product was collected by vacuum filtration to give 0.8 g (7%) of 5-methoxycarbonyl-2-oxindole as an off-white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.70 (s, br, 1H, NH-1), 7.83 (dd, J = 1.77, 8.29 Hz, 1H, H-6), 7.77 (s, br, 1H, H-4), 6.89

20   (d, J = 8.29 (Hz, 1H, H-7), 3.80 (s, 3H, COOCH$_3$-5), 3.51 (s, 2H, CH$_2$-3).

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5-methoxycarbonyl-2-oxindole to give 0.25 g of 3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-

25   5-carboxylic acid methyl ester as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.96 (s, 1H, CONH), 8.29 (d, J = 1Hz, 1H, H-4), 7.88 (dd, J = 1,8Hz, 1H, H-6), 7.7 (s, 1H, =CH-), 7.55 (s, 2H, aromatic), 7.0 (d, J = 8Hz, 1H, H-7), 3.76 (s, 3H, OCH$_3$), 3.73 (s, 3H, OCH$_3$), 3.26-3.35 (m, 2H), 1.23 (d, J =

30   7Hz, 12H, 4xCH$_3$).

MS m/z 393.

Example 7:   **3-(3-Isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one**

2-Isopropylphenol was methylated and then formylated to give 3-isopropyl-4-methoxybenzaldehyde. 3-Isopropyl-4-methoxybenzaldehyde was condensed with 2-oxindole to give 0.3 g of 3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-

5 one as a yellow-orange solid.

^{1}HNMR (d_6-DMSO) δ: 10.5 (s, 1H, CONH), 7.7, 7.6, 7.2, 7.1, 6.8 (multiplets, 8H, aromatic, =CH-), 3.9 (s, 3H, OCH$_3$), 3.2 (m, 1H, CH), 1.2 (d, 6H, 2xCH$_3$).

Example 8:   3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-

10 **1,3-dihydroindol-2-one**

5-Isopropyl-4-methoxy-2-methylbenzaldehyde was condensed with 2-oxindole to give 0.25 g of 3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

15   ^{1}HNMR (d_6-DMSO) δ: 10.5 (s, 1H, CONH), 7.6, 7.4, 7.3, 7.2, 6.9, 6.8, 6.8 (multiplets, 7H, aromatic, =CH-), 3.9 (s, 3H, OCH$_3$), 3.2 (m, 1H, CH), 2.3 (s, 3H, CH$_3$), 1.2 (d, 6H, 2xCH$_3$).

Example 9:   5-Chloro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one

20   2-Isopropyl-5-methylphenol was methylated and then formylated to give 5-isopropyl-4-methoxy-2-methylbenzaldehyde.

^{1}HNMR (d_6-DMSO) δ: 10.1 (s, 1H, CHO), 7.6, 6.9 (2xs, 2H, aromatic), 3.9 (s, 3H, OCH$_3$), 3.2 (m, 1H, CH), 2.6 (s, 3H, CH$_3$), 1.2 (d, 6H, 2xCH$_3$).

25   5-Isopropyl-4-methoxy-2-methylbenzaldehyde was condensed with 5-chloro-2-oxindole to give 0.3 g of 5-Chloro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (d_6-DMSO) δ: 10.65 (s, 1H, CONH), 7.73 (s, 1H,),

30 7.49 (s, 1H,), 7.4 (d, J = 2Hz, 1H, H-4), 7.23 (dd, J = 2,8Hz, 1H, H-6), 6.98 (s, 1H), 6.86 (d, J = 8Hz, 1H, H-7), 3.86 (s, 3H, OCH$_3$), 3.23-3.3 (m, 2H), 2.33 (s, 3H, CH$_3$), 1.15 (d, J = 7Hz, 6H, 2xCH$_3$).

MS m/z 341.

Example 10:   3-(3-Cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-Cyclopentyl-4-methoxybenzaldehyde was condensed with 2-
5   oxindole to give 0.25 g of 3-(3-Cyclopentyl-4-
methoxybenzylidene)-1,3-dihydro-indol-2-one as a yellow-orange
solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.5 (s, 1H, CONH), 7.6, 7.5, 7.2, 7.1,
6.8 (multiplets, 8H, aromatic, =CH-), 3.9 (s, 3H, OCH$_3$), 3.2
10   (m, 1H, CH), 1.8 (multiplets, 8H, 4xCH$_2$).

Example 11:   3-(3-Cyclopentyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one

2-Cyclopentylphenol was methylated and then formylated to
give 3-cyclopentyl-4-methoxybenzaldehyde.

15   ^{1}HNMR (d$_6$-DMSO) δ: 9.9 (s, 1H, CHO), 7.8, 7.7, 7.2
(multiplets, 3H, aromatic), 3.9 (s, 3H, OCH$_3$), 3.2 (m, 1H, CH),
1.2 (multiplets, 8H, 4xCH$_2$).

3-Cyclopentyl-4-methoxybenzaldehyde was condensed with 5-
fluoro-2-oxindole to give 0.25 g of 3-(3-cyclopentyl-4-
20   methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one as a
yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.5 (s, 1H, CONH), 7.6, 7.6, 7.6, 7.4,
7.1, 7.0, 6.8 (multiplets, 7H, aromatic, vinyl CH), 3.9 (s,
3H, CH$_3$), 3.3 (m, 1H, CH), 2.0-1.5 (multiplets, 8H, 4xCH$_2$).
25   MS m/z 337.

Example 12:   3-(3-Cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

2-Cyclohexylphenol was methylated and then formylated.

The aldehyde was condensed with oxindole to give 0.3 g of
30   3-(3-Cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.5 (s, 1H, CONH), 7.7, 7.6, 7.6, 7.2,
7.1, 6.8, 6.8 (multiplets, 7H, aromatic, =CH-), 3.9 (s, 3H,

OCH₃), 3.0 (m, 1H, CH), 1.8, 1.4 (multiplets, 10H, 5xCH₂).

Example 13: 5-Bromo-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

2-Phenylanisole was formylated to give 4-methoxy-3-phenylbenzaldehyde.

¹HNMR (d₆-DMSO) δ: 9.9 (s, 1H, CHO), 7.9, 7.8, 7.5, 7.4, 7.4, 7.3 (multiplets, 8H, aromatic), 3.9 (s, 3H, OCH₃.

MS m/z 213.

4-Methoxy-3-phenylbenzaldehyde was condensed with 5-bromo-2-oxindole to give 0.3 g of 5-Bromo-3-(6-methoxy-biphenyl-3-ylmethylene)-1,3-dihydroindol-2-one as a yellow-orange solid.

¹HNMR (d₆-DMSO) δ: 10.6 (s, 1H, CONH), 7.8-6.8 (multiplets, 12H, aromatic, =CH-), 3.9 (s, 3H, OCH₃).

Example 14: 5-Chloro-3-(2,3-dihydro-benzofuran-5-ylmethylene)-1,3-dihydroindol-2-one

2,3-Dihydro-5-formylbenzofuran was condensed with 5-chloro-2-oxindole to give 0.25 g of 5-Chloro-3-(2,3-dihydro-benzofuran-5-ylmethylene)-1,3-dihydroindol-2-one as a yellow-orange solid.

¹HNMR (d₆-DMSO) δ: 10.6 (s, 1H, CONH), 8.6, 8.2, 7.7, 7.6, 7.2, 6.9, 6.8 (multiplets, 7H, aromatic, =CH-), 4.6 (t, 2H, OCH₂), 3.2 (t, 2H, OCH₂).

Example 15: 5-Chloro-3-(2,2-dimethyl-chroman-6-ylmethylene)-1,3-dihydroindol-2-one

2,2-Dimethyl-6-formylchromane was condensed with 5-chloro-2-oxindole to give 0.3 g of 5-Chloro-3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one as a yellow-orange solid.

¹HNMR (d₆-DMSO) δ: 10.61 (s, 1H, CONH), 8.31, 8.27, 7.76, 7.17, 6.79 (multiplets, 6H, aromatic), 7.8 (s, 1H, =CH-), 2.78 (t, 2H, CH₂), 1.81 (t, 2H, CH₂), 1.31 (s, 6H, 2xCH₃).

MS m/z 340.5 (M+1).

Example 16: <u>N-{3-[3-Cyclohexyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide</u>

Triphenylphosphine (7.47 g, 19.58 mmol) was added to a solution of 2-cyclohexyl-4-chlorophenol (6 g, 28.48 mmol) and

5   2-hydroxyethylmorpholine (3.5 g, 28.48 mmol) in tetrahydrofuran (50 ml), followed by the dropwise addition of diethylazodicarboxylate (4.5 ml, 28.48 mmol). The mixture stirred at room temperature for 12 hours. The reaction mixture was concentrated under reduced pressure and triturated with

10   dichloromethane/ hexanes and then washed with more hexanes. Chromatography (silica, 10-20-30% ethyl acetate/hexanes) afforded 8.4 g (95%) of 3-cyclohexyl-4-morpholinoethoxy-chlorobenzene as a yellowish oil.

^{1}HNMR (360 MHz, DMSO-d6) δ: 7.12 (m, 2H, 2xAr-H), 6.93 (d,

15   J = 9Hz, 1H, Ar-H), 4.04 (m, 2H, O-CH$_2$CH$_2$), 3.55 (m, 4H, 2x ringO-CH$_2$CH$_2$), 2.84 (m, 1H, CHcyclohexyl), 2.68 (m, 2H, N-CH$_2$CH$_2$), 2.47 (m, 4H, 2x ringN-CH$_2$CH$_2$), 1.7 (m, 5H, cyclohexyl), 1.3 (m, 5H, cyclohexyl).

MS m/z 324.4 and 325.4, [M+1]$^+$ and [M+3]$^+$.

20   Naphthalene (3.48 g, 27.2 mmol) was added to a suspension of 30% lithium dispersion (3.2 g, 138 mmol, pre-washed with tetrahydrofuran) in tetrahydrofuran (80 ml). The suspension was stirred until a green color appeared (approximately 1 hour). The reaction was cooled to -78°C, and a solution of the

25   3-cyclohexyl-4-morpholinoethoxychlorobenzene (8 g, 24.7 mmol) in tetrahydrofuran (20ml) was added. After 1 hour, the reaction was warmed to 0°C and stirred for an additional hour. Dimethylformamide (9.6 ml, 123.5 mmol) was added, the reaction was stirred at 0°C for one more hour and then warmed to room

30   temperature and stirred for another hour. The reaction was quenched with methanol (30 ml), added to 1N hydrochloric acid (300 ml) and extracted with ethyl acetate (400 ml). The organic layer was washed with water (300 ml), saturated sodium

bicarbonate solution (300 ml) and brine (300 ml), dried over
magnesium sulfate and concentrated. Chromatography (silica,
40-60% ethyl acetate/hexanes) afforded 3 g (34%) of 3-
cyclohexyl-4-morpholinoethoxybenzaldehyde as a light yellow
5   oil.

^{1}HNMR (360 MHz, DMSO-d6) δ: 9.83 (s, 1H, CHO), 7.12 (m,
2H, 2xAr-H), 7.13 (d, J = 8Hz, 1H, Ar-H), 4.19 (m, 2H, O-
CH$_2$CH$_2$), 3.55 (m, 4H, 2x ringO-CH$_2$CH$_2$), 2.88 (m, 1H,
CHcyclohexyl), 2.74 (m, 2H, N-CH$_2$CH$_2$), 2.49 (m, 4H, 2x ringN-
10   CH$_2$CH$_2$), 1.7 (m, 5H, cyclohexyl), 1.3 (m, 5H, cyclohexyl).
MS m/z 318.4 [M+1]$^+$.

A mixture of 3-cyclohexyl-4-morpholinoethoxybenzaldehyde
(3 g, 9.45 mmol), 6-acetamido-2-oxindole (3.5g, 18.9 mmol) and
piperidine (5 ml, 50 mmol) in ethanol (35 ml) was held in a
15   sealed tube at 100°C for 6 hours. The reaction mixture was
cooled, diluted with ethyl acetate (500 ml) and filtered to
remove excess 6-acetamido-2-oxindole. The filtrate was
extracted with 0.5N hydrochloric acid (200 ml) and brine (2 X
200 ml). The acid wash was basified with solid sodium
20   bicarbonate to pH 9 and extracted with ethyl acetate (2 x 200
ml). The combined organic layers were washed with brine (200
ml), dried over magnesium sulfate and concentrated. The
combined solids were dissolved in dichloromethane (25 ml) and
precipitated with diethyl ether (500 ml) to afford 1.6g of a
25   mustard yellow solid. The solid was dissolved in methanol
(100 ml) and filtered, affording a yellow solid (which was set
aside) and then precipitated with water (400 ml) and filtered.
The solid residue was redissolved in methanol and
concentrated to a solid. This process was repeated twice to
30   afford ~82% pure isomer 1 by HPLC. The filtered yellow solid
was 77% isomer 2 by HPLC.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.45 (s, 1H, NH), 10.0 (s,
1H, NHAc), 7.55 (m, 3H, Ar-H), 7.44 (m, 2H, Ar-H and Ar-CH=C),

7.07 (d, 1H, J = 8Hz, Ar-H), 6.89 (dd, 1H, J =2 and 8Hz, Ar-H), 4.16 (m, 2H, Ar-OCH$_2$), 3.57 (m, 4H, OCH$_2$CH$_2$N), 2.92 (m, 1H, chexCH), 2.75 (m, 2H, ArOCH$_2$-CH$_2$N), 2.51 (m, 4H, NCH$_2$CH$_2$O), 2.02 (s, 3H, NHCOCH$_3$), 1.8 (m, 5H, chexCH$_2$), 1.38 (m, 5H, chexCH$_2$).

20% isomer 1; 77% isomer 2

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.39 (s, 1H, NH), 9.92 (s, 1H, NHAc), 8.37 (d, 1H, J = 2Hz, Ar-H), 8.24 (m, 1H, Ar-H), 7.55 (s, 1H, Ar-CH=C), 7.52 (d, 1H, J = 8Hz, Ar-H), 7.36 (m, 1H, Ar-H), 7.03 (m, 2H, Ar-H), 4.16 (m, 2H, Ar-OCH$_2$), 3.57 (m, 4H, OCH$_2$CH$_2$N), 2.88 (m, 1H, chexCH), 2.74 (m, 2H, ArOCH$_2$-CH$_2$N), 2.51 (m, 4H, NCH$_2$CH$_2$O), 2.03 (s, 3H, NHCOCH$_3$), 1.8 (m, 5H, chexCH$_2$), 1.38 (m, 5H, chexCH$_2$).

Example 17:   **3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-methoxy-1,3-dihydroindol-2-one**

3,5-Diisopropyl-4-hydroxybenzaldehyde was methylated to give 3,5-diisopropyl-4-methoxybenzaldehyde.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.1 (s, 1H, CHO), 6.9 (s, 2H, aromatic), 3.9 (s, 3H, OCH$_3$), 3.2 (m, 2H, 2xCH), 1.2 (d, 12H, 4xCH$_3$).

A solution of 11.4 g of hydroxylamine hydrochloride in water (50 ml) was added to a solution of chloral hydrate (9.6 g) and sodium sulfate (83 g) in water (200 ml) at 60°C. The mixture was held at 60°C while, in a separate flask, a solution of 4-anisidine (6.4 g) and concentrated hydrochloric acid (4.3 ml) in water (80 ml) was warmed to 80°C. The first solution was then added to the second and the reaction was refluxed for 2 minutes, cooled slowly to room temperature and then cooled in an ice bath. The tan precipitate which formed was collected by vacuum filtration, washed with water and dried under vacuum to give 8.6 g (85 % yield) of N-(2-hydroximinoacetyl)anisidine.

Concentrated sulfuric acid (45 ml) containing water (5 ml) was warmed to 60°C and 8.6 g of N-(2-hydroximinoacetyl)-anisidine was added in one portion. The mixture was stirred

at 93°C for 10 minutes and then allowed to cool to room
temperature. The mixture was poured into 500 g of ice and
extracted 3 times with ethyl acetate. The combined extracts
were dried over anhydrous sodium sulfate and concentrated to
5   give 5.1 g (65 % yield) of 5-methoxyisatin as a dark red
solid.

A mixture of 5-methoxyisatin (5.0 g) and 30 ml of
hydrazine hydrate was refluxed for 15 minutes. The reaction
mixture was cooled to room temperature and 50 ml of water was
10 added. The mixture was extracted 3 times with 25 ml of ethyl
acetate, the organic layers combined, dried over anhydrous
sodium sulfate and concentrated to give a yellow solid. The
solid was stirred in ethyl acetate and 1.1 g of insoluble
material removed by vacuum filtration and saved. This
15 material proved to be 2-hydrazinocarbonylmethyl-4-anisidine.
The filtrate was concentrated and chromatographed on silica
gel eluting with ethyl acetate:hexane 1:1 to give 0.7 g of 5-
methoxy-2-oxindole as a dirty yellow solid. The 1.1 g of 2-
hydrazinocarbonylmethyl-4-anisidine was refluxed for 1 hour in
20 20 ml of 1 N sodium hydroxide. The mixture was cooled,
acidified to pH 2 with concentrated hydrochloric acid and
extracted 3 times with 25 ml of ethyl acetate. The organic
extracts were combined, washed with brine, dried over
anhydrous sodium sulfate and concentrated to give 0.8 g of 5-
25 methoxy-2-oxindole as a dirty yellow solid. The combined
yield was 1.5 g or 33 %.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.13 (s, 1H, NH-1), 6.84 (s,
1H, H-4), 6.72 (d, J = 9Hz, 1H, H-6), 6.69 (d, J = 9Hz, 1H, H-
7), 3.68 (s, 3H, OCH$_3$-5), 3.41 (s, 2H, CH$_2$-3).
30   MS m/z 163 [M+1]$^+$.

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with
5-methoxy-2-oxindole to give 0.3 g of 3-(3,5-Diisopropyl-4-
methoxybenzylidene)-5-methoxy-1,3-dihydroindol-2-one as a

yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.34 (s, 1H, NH), 7.59 (s, 2H), 7.47 (s, 2H), 7.15 (d, J = 2Hz, 1H), 6.85 (dd, J = 2, 8Hz, 1H), 6.58 (d, J = 8Hz, 1H), 3.73 (s, 3H, OCH$_3$), 3.63 (s, 3H, OCH$_3$), 3.25-3.39 (m, 2H, 2 x (CH$_3$)$_2$CH), 1.2 (d, 12H, 2 x (CH$_3$)$_2$CH).

MS APCI m/z 366.1 [M+1]$^+$.

Example 18:   **N-[3-(4-Methoxy-3-thiophene-3-ylbenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide**

Tetrakis(triphenylphosphine)palladium(0) (1.35 g) was added to a solution of 4-methoxy-3-bromobenzaldehyde (6.72 g) in toluene (45 ml) and ethanol (45 ml), followed by addition of 2M aqueous sodium carbonate (80 ml). To this mixture was added thiophene-3-boronic acid (5 g). The mixture was then refluxed for 12 hours. The reaction mixture was poured into water (200 ml) and extracted into ethyl acetate (2 x 150 ml). The organic layer was washed with saturated aqueous sodium bicarbonate (150 ml) and brine (150 ml), dried over magnesium sulfate and concentrated. Chromatography (silica, 20% ethyl acetate/hexanes) afforded 6 g (88%) of 3(3-thiophene)-4-methoxybenzaldehyde as a yellow oil.

^{1}HNMR (360 MHz, DMSO-d6) δ: 9.92 (s, 1H, CHO), 8.04 (d, 1H, J = 2Hz, 1xAr-H), 7.8 (m, 2H, Ar-H), 7.58 (dd, 1H, J = 3 and 5Hz, thiophene and Ar-H), 7.50 (dd, 1H, J = 2 and 5Hz, Ar-H), 7.28 (d, 1H, J = 9Hz, thiophene), 3.94 (s, 3H, OCH$_3$).

MS m/z 219.2 [M+1]$^+$.

A mixture of 3(3-thiophene)-4-methoxybenzaldehyde (1.72 g), 6-acetamido-2-oxindole (1.5g) and piperidine (4 ml) in ethanol (26 ml) was held in a sealed tube for 12 hours at 100°C. The reaction was cooled and poured into diethyl ether. The precipitate which formed was removed by filtration, washed with diethyl ether and then hexanes and dried to afford 0.9 g (29%) of N-[3-(4-methoxy-3-thiophene-3-ylbenzylidene)-2-

oxo-2,3-dihydro-1H-indol-6-yl]-acetamide as a tan solid.

^{1}HNMR 360 MHz, DMSO-d6) δ: 10.52 (s, 1H, NH), 10.22 (s, 1H, NHAc), 7.85 (m, 2H, 2xAr-H), 7.7 (dd, J = 2 and 9Hz, 1H, Ar-H), 7.6 (m, 2H, 2xAr-H), 7.5 (dd, 1H, J = 1 and 5Hz, Ar-H), 7.47 (s, 2H, Ar-H and Ar-CH=C), 7.25 (d, 1H, J =9Hz, Ar-H), 6.96 (dd, 1H, J =2 and 9Hz, Ar-H), 3.92 (s, 3H, OCH$_3$), 2.04 (s, 3H, NHCOCH$_3$).

MS m/z 391.2 [M+1]$^+$.

Example 19:   3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-dihydroindol-2-one

A mixture of 5-methylisatin (15.0 g) and 60 ml of hydrazine hydrate was stirred at 140 - 160°C for 4 hours. The reaction mixture was cooled to room temperature, poured into 300 ml of ice water and acidified to pH 2 with 6 N hydrochloric acid. After standing at room temperature for 2 days, a precipitate formed which was collected by vacuum filtration, washed with water and dried under vacuum to give 6.5 g (47 % yield) of 5-methyl-2-oxindole.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.20 (s, br, 1H, NH-1), 6.99 (s, 1H, H-4), 6.94 (d, J = 8 Hz, 1H, H-6), 6.68 (d, J = 8Hz, 1H, H-7), 3.39 (s, 2H, CH$_2$-3), and 2.22 (s, 3H, CH$_3$-5).

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5-methyl-2-oxindole to give 0.3 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.41 (s, br, 1H, NH), 7.56 (s, 1H), 7.51 (s, 2H), 7.50 (s, br, 1H), 7.03 (d, br, J = 8Hz, 1H), 6.76 (d, J = 8Hz, 1H), 3.74 (s, 3H, OCH$_3$), 3.26-3.35 (m, 2H, 2 x (CH$_3$)$_2$CH), 2.15 (s, 3H, CH$_3$), 1.22 (d, J = 7Hz, 12H, 2 x (CH$_3$)$_2$CH).

MS APCI m/z 350.2 [M+1]$^+$.

Example 20:   5-Amino-3-(3,5-diisopropyl-4-methoxy-benzylidene)-1,3-dihydroindol-2-one

2-Oxindole (6.5 g) was dissolved in concentrated sulfuric acid (25 ml) and the mixture maintained at -10 to 15°C while fuming nitric acid (2.1 ml) was added dropwise. After the addition of the nitric acid the reaction mixture was stirred

5   at 0°C for 0.5 hour and poured into ice-water. The precipitate which formed was collected by filtration, washed with water and crystallized from 50% acetic acid. The final crystalline product was then filtered, washed with water and dried under vacuum to give 6.3 g (70%) of 5-nitro-2-oxindole.

10   5-Nitro-2-oxindole (6.3 g) was hydrogenated in methanol over 10% palladium on carbon to give 3.0 g (60% yield) of 5-amino-2-oxindole as a white solid.

         3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5-amino-2-oxindole to give 0.3 g of 5-Amino-3-(3,5-

15   diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

         ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.62 (br s, 1H, NH), 8.31 (s, 2H), 7.8 (s, 1H), 7.65 (s, 1H), 7.55 (d, 1H), 7.47 (s, 1H), 7.09 (dd, J = 2, 8Hz, 1H), 6.88 (d, J = 8Hz, 1H), 3.72 (3H,

20   OCH$_3$), 3.24-3.32 (m, 2H, 2 x (CH$_3$)$_2$CH), 1.23 (d, J = 6.5Hz, 12H, 2 x (CH$_3$)$_2$CH).

Example 21:   <u>5-Chloro-3-(4-methoxy-3,5-dimethylbenzylidene)-</u>
<u>1,3-dihydroindol-2-one</u>

         2,6-Dimethylphenol was methylated and then formylated.

25   ^{1}HNMR (d$_6$-DMSO) δ: 10.1(s, 1H, CHO), 6.9(s, 2H, aromatic), 3.9(s, 3H, OCH$_3$), 2.6(s, 6H, 2xCH$_3$).

         3,5-Dimethyl-4-methoxybenzaldehyde was condensed with 5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-dihydroindol-2-one as a yellow-orange

30   solid.

Example 22:   <u>3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-</u>
<u>fluoro-1,3-dihydroindol-2-one</u>

         A suspension of sodium hydride (2.6 g) and

dimethylmalonate (14.5 g) in dimethylsulfoxide (160 ml) was
stirred at 100°C for 1 hour. The mixture was cooled to room
temperature and 7.95 g of 2,5-difluoronitrobenzene was added
and stirring was continued for 30 minutes. The mixture was
5   then heated to 100°C and stirred for 1 additional hour, then
cooled to room temperature and poured into 400 ml of saturated
ammonium chloride. The mixture was extracted with ethyl
acetate (200 ml) and the organic layer washed with brine,
dried over anhydrous sodium sulfate and concentrated under
10   vacuum. The residue was crystallized from methanol to give
24.4 g (80% yield) of dimethyl 4-fluoro-2-nitrophenylmalonate
as a white solid. The filtrate was concentrated and
chromatographed on a column of silica gel (ethyl
acetate:hexane 1:8) to give 5.03 g of dimethyl 4-fluoro-2-
15   nitrophenylmalonate, for a total of 29.5 g (96 % yield).

Dimethyl 4-fluoro-2-nitrophenylmalonate (5.0 g) was
refluxed in 20 ml of 6 N hydrochloric acid for 24 hours. The
reaction was cooled and the white solid which formed was
collected by vacuum filtration, washed with water and dried to
20   give 3.3 g (87% yield) of 4-fluoro-2-nitrophenylacetic acid.

4-Fluoro-2-nitrophenylacetatic acid (3.3 g) dissolved in
acetic acid (15 ml) was hydrogenated over 0.45 g of 10%
palladium on carbon under 60 psi for 2 hours. The catalyst
was removed by filtration and washed with 15 ml of methanol.
25   The combined filtrates were concentrated and diluted with
water. The precipitate was collected by vacuum filtration,
washed with water and dried to give 1.6 g (70% yield) of 6-
fluoro-2-oxindole. The filtrate was concentrated to give a
purple solid with an NMR spectrum similar to the first crop.
30   Chromatography of the purple solid (ethyl acetate:hexane 1:2,
silica gel) gave a second crop of 6-fluoro-2-oxindole as a
white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.43 (s, 1H, NH-1), 7.17 (t,

J = 8Hz, H-4), 6.69 (ddd, *J* = 2, 8, 10Hz, 1H, H-5), 6.6 (dd, *J*

= 2, 9Hz, 1H, H-7), and 3.42 (s, 2H, CH₂-3).

MS m/z 152.8 [M+1]⁺.

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with

5   6-fluoro-2-oxindole to give 0.3 g of 3-(3,5-diisopropyl-4-

methoxybenzylidene)-6-fluoro-1,3-dihydroindol-2-one as a

yellow-orange solid.

¹HNMR (360 MHz, DMSO-d6) δ: 10.69 (s, br, 1H, NH), 7.58

(s, 1H, vinyl), 7.55-7.59 (m, 1H), 7.47 (s, 2H), 6.68 (m, 2H),

10   3.73 (s, 3H, OCH₃), 3.25-3.34 (m, 2H, 2 x (CH₃)₂CH), 1.2 (d, *J*

= 7Hz, 12H, 2 x (CH₃)₂CH).

MS   m/z 353.

Example 23:   **3-(2,2-Dimethylchroman-6-ylmethylene)-5-fluoro-**
1,3-dihydroindol-2-one

15   2,2-Dimethyl-6-formylchromane (commercially available)

was condensed with 5-fluoro-2-oxindole to give 0.3 g of 3-

(2,2-Dimethylchroman-6-ylmethylene)-5-fluoro-1,3-dihydroindol-

2-one as a yellow-orange solid.

Example 24:   **5-Chloro-3-[3,5-diisopropyl-4-(2-morpholin-4-**
20 **ylethoxy)-benzylidene]-1,3-dihydroindol-2-one**

Triphenylphosphine (5.14 g, 19.58 mmol) was added to a

solution of 3,5-diisopropyl-4-hydroxybenzaldehyde (4 g, 19.58

mmol) in tetrahydrofuran (40 ml) followed by addition of 2-

hydroxyethylmorpholine (2.57 g, 19.58 mmol) and then the

25 dropwise addition of diethylazodicarboxylate (3.41 g, 19.58

mmol). The mixture was stirred at room temperature for 12

hours. The reaction was concentrated under reduced pressure

and partitioned between 2N hydrochloric acid (200 ml) and

ethyl acetate (150 ml). The aqueous layer was extracted with

30 ethyl acetate (2 x 150ml), basified to pH 9 with solid sodium

bicarbonate and extracted with ethyl acetate (3 x 150 ml). The

organic layers were combined, dried over magnesium sulfate and

concentrated to afford 900 mg (14%) of 3,5-diisopropyl-4-(2-

morpholin-4-ylethoxy)-benzaldehyde as a yellowish oil.

^{1}HNMR (360 MHz, DMSO-d6) δ: 9.91 (s, 1H, CHO), 9.37 (s, 2H, 2xAr-H), 3.85 (t, J = 5Hz, 2H, O-CH$_2$CH$_2$), 3.59 (m, J = 5 and 4Hz, 4H, 2x ringO-CH$_2$CH$_2$), 3.39 (sept, J = 7Hz, 2H, 2xCHCH$_3$), 2.72 (t, J = 5Hz, 2H, N-CH$_2$CH$_2$), 2.4 (m on DMSO, 4H, 2x ringN-CH$_2$CH$_2$), 1.2 (d, J = 7Hz, 12H, 4xCH$_3$).

A mixture of 3,5-diisopropyl-4-(2- morpholin-4-ylethoxy)-benzaldehyde (0.4 g, 1.26 mmol), 5-chloro-2-oxindole (0.21g, 1.26 mmol) and pyrrolidine (0.5 ml, 6.3 mmol) in ethanol (2 ml) was held in sealed tube at 100°C for 12 hours. The mixture was then poured into 1N hydrochloric acid (100 ml) and the solid which remained were filtered and washed with more water (50 ml). The solid was then dissolved in ethyl acetate (200 ml), the solution dried over magnesium sulfate, fiiltered and then concentrated. The solid which remained was chromatographed (silica, 4/4/1 dichloromethane/ hexanes/ methanol) to give 60 mg (10%) of 5-chloro-3-[3,5-diisopropyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-1,3-dihydroindol-2-one as a brownish-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.54 (s, 1H, NH), 9.43 (d, J = 10Hz, 1H, Ar-H), 7.58 (d, J = 9Hz, 1H, Ar-H), 7.46 (d, J = 5Hz, 2H, Ar-H), 7.3 (m, 1H, Ar-H), 7.19 (m, J = 9 and 10Hz, 1H, Ar-H), 6.99 (m, J = 9 and 9Hz, 1H, Ar-H), 6.83 (m, J = 9 and 1Hz, 1H, Ar-H), 3.61 (dd, J = 9 and 22 HZ 1H, 1xCH$_2$CH), 3.43 (m, 1H, CHCH$_3$), 2.86 (dd, J = 4 and 22Hz, 1H, 1xCH$_2$CH), 1.35 (d, J = 9Hz, 3H, CH$_3$).

MS m/z 262.0 [M+1]$^+$.

Example 25:   3-(3,5-Diisopropyl-4-methoxybenzylidene)-7-fluoro-1,3-dihydroindol-2-one

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 7-fluoro-2-oxindole to give 0.25 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-7-fluoro-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 26:   3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-5-(2-
morpholin-4-yl-ethyl)-1,3-dihydroindol-2-one

A solution of 5-chloroacetyl-2-oxindole (4.18 g) in
trifluoroacetic acid (30 ml) was cooled in an ice bath and

5   4.65 g of triethylsilane added. The mixture was then stirred
at room temperature for 3 hours. The mixture was poured into
150 ml of water and the precipitate which formed was collected
by vacuum filtration, washed with 50 ml of water and dried to
give 2.53 g (65 % yield) of 5-(2-chloroethyl)-2-oxindole as a

10   reddish-brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.26 (s, br, NH), 7.11 (s,
1H, H-4), 7.05 (d, J = 8Hz, 1H), 6.73 (d, J = 8Hz, 1H), 3.77
(t, J = 7Hz, 2H, CH$_2$), 3.42 (s, 2H, CH$_2$), 2.94 (t, J = 7Hz, 2H,
CH$_2$).

15   A solution of 5-(2-chloroethyl)-2-oxindole (2.3 g),
morpholine (1.2 ml) and diisopropylethylamine (1.2 ml) in
dimethylsulfoxide (10 ml) was heated overnight at 100°C. The
mixture was cooled, poured into water and extracted with ethyl
acetate. The organic layer was washed with brine, dried and

20   evaporated. The residue was chromatographed (silica gel, 5 %
methanol in chloroform) to give 0.9 g (31 %) of 5-(2-
morpholin-4-yl-ethyl)-2-oxindole as a white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.21 (s, br, 1H, NH), 7.06
(s, br, 1H, H-4), 7.0 (d, J = 8Hz, 1H, H-6), 6.71 (d, J = 8Hz,

25   1H, H-7), 3.55-3.58 (m, 4H), 3.41 (s, 2H, H-3), 2.63-2.68 (m,
2H), 2.39-2.47 (m, 6H).

A mixture of 3(3-thiophene)-4-methoxybenzaldehyde (0.27
g, 1.22 mmol), 5-(2-morpholin-4-yl-ethyl)-2-oxindole (0.3g,
1.22 mmol) and piperidine (0.6 ml, 6.1 mmol) in ethanol (4 ml)

30   was held in a sealed tube at 100°C for 12 hours. The reaction
was cooled and poured into diethyl ether (150 ml) and hexanes
(150 ml). The solid which remained was removed by filtration,
washed with diethyl ether and then hexanes and dried to afford

0.27 g (50%) of 3-(4-methoxy-3-thiophen-3-ylbenzylidene)-5-(2-morpholin-4-yl-ethyl)-1,3-dihydroindol-2-one as an orange-yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.41 (s, 1H, NH), 8.86 (d, J = 2.15Hz, 1H, Ar-H), 8.39 (dd, J = 2 and 9Hz, 1H, Ar-H), 7.8 (dd, 1H, J = 1 and 3Hz, Ar-H), 7.7 (s, 1H, Ar-CH=C), 7.6 (m, 1H, ArH), 7.5 (m, 2H, 2xArH), 7.21 (d, J = 9Hz, 1H, Ar-H), 7.02 (dd, 1H, J = 1 and 8Hz, Ar-H), 6.73 (d, 1H, J = 8Hz, Ar-H), 3.93 (s, 3H, OCH$_3$), 3.58 (m, 4H, 2x OCH$_2$CH$_2$N), 2.7 (m, 2H, ArCH$_2$CH$_2$), 2.5 (m, 2H, ArCH$_2$CH$_2$N), 2.4 (m, 4H, 2xOCH$_2$CH$_2$N).

MS m/z 447.2 [M+1]$^+$.

Example 27: _N-[3-(5-Isopropyl-4-methoxy-2-methyl-benzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide_

A mixture of 5-isopropyl-4-methoxy-2-methylbenzaldehyde (0.25 g, 1.3 mmol), 6-acetamido-2-oxindole (0.25g, 1.3 mmol) and pyrrolidine (0.54 ml, 6.5 mmol) in ethanol (4 ml) was held in a sealed tube at 100°C for 12 hours. The reaction was cooled and then poured into water (100 ml). The solid which formed was removed by filtration, washed with water and then dissolved in ethyl acetate (200 ml). The ethyl acetate solution was dried over magnesium sulfate and concentrated. The resulting solid was triturated with dichloromethane/hexanes to afford 0.33 g (70%) of N-[3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide as a brownish yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.46 (s, 1H, NH), 7.49 (s, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 7.41 (s, 1H, Ar-H), 6.93 (m, 1H, Ar-CH=C), 6.83 (dd, 1H, J =2 and 8Hz, Ar-H), 3.83 (s, 3H, OCH$_3$), 2.30 (s, 3H, Ar-CH$_3$), 2.02 (s, 3H, NHCOCH$_3$), 1.2 (m, 1H, CH(CH$_3$)$_2$), 1.1 (d, J = 7Hz, 6H, CH(CH$_3$)$_2$).

MS m/z 365.2 [M+1]$^+$.

Example 28: _3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-ethyl-1,3-dihydroindol-2-one_

2-Oxindole (3 g) suspended in 1,2-dichloroethane was slowly treated with 3.2 ml of acetyl chloride. The resulting suspension was stirred at 50°C for 5 hours, cooled, and poured into water. The resulting precipitate was collected by vacuum

5   filtration, washed copiously with water and dried under vacuum to give 2.9 g (73 % yield) of 5-acetyl-2-oxindole as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ : 10.75 (s, br, NH), 7.83 (d, J = 8Hz, 1H), 7.78 (s, 1H, H-4), 6.88 (d, J = 8Hz, 1H), 3.53 (s,

10   2H, CH_2), 2.49 (s, 3H, CH_3).

5-Acetyl-2-oxindole (2 g) and 15 ml of trifluoroacetic acid in an ice bath was slowly treated with 1.8 g of triethylsilane and then stirred at room temperature for 5 hours. One ml of triethylsilane was added and stirring

15   continued overnight. The reaction mixture was poured into ice water and the resulting precipitate collected by vacuum filtration, washed copiously with water and dried under vacuum to give 1.3 g (71 % yield) of 5-ethyl-2-oxindole as a yellow solid.

20   ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.25 (s, br, NH-1), 7.03 (s, 1H, H-4), 6.97 (d, J = 8Hz, 1H, H-6), 6.69 (d, J = 8Hz, 1H, H-7), 3.40 (s, 2H, CH_2-3), 2.51 (q, J = 8Hz, 2H, CH_2CH_3-5), and 1.12 (t, J = 7Hz, 3H, CH_2CH_3-5).

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with

25   5-ethyl-2-oxindole to give 0.3 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-5-ethyl-1,3-dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.42 (s, 1H, NH), 7.56 (s, 1H), 7.51 (s, 2H), 7.49 (s, br, 1H), 7.08 (dd, J = 8Hz, 1H),

30   6.79 (d, J = 8Hz, 1H), 3.74 (s, 3H, OCH3), 3.25-3.55 (m, 2H, 2 x $(CH_3)_2CH$), 2.44 (q, J = 8Hz, 2H, CH_3CH_2), 1.22 (d, J = 7Hz, 12H, 2 x $(CH_3)_2CH$), 1.08 (t, J = 8Hz, 3H, CH_3CH_2).

MS APCI m/z 364.3 [M+1]$^+$.

Example 29: _N-[2'-Methoxy-5'-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-biphenyl-3-yl]-acetamide_

A mixture of 3-bromo-p-anisaldehyde (1 g, 4.65 mmol),
pyrrolidine (1.9 ml, 23.2 mmol) and oxindole (0.62 g, 4.65

5 mmol) in dimethylformamide (7 ml) was held in a sealed tube at
100°C for 12 hours. The mixture was cooled to room temperature
and poured into 1N hydrochloric acid (100 ml). The precipitate
which formed was filtered and washed with water. The
precipitate was then dissolved in ethyl acetate (200 ml), the

10 solution washed with brine, dried over magnesium sulfate and
then concentrated. The solid obtained was chromatographed
(silica, 30-40% ethyl acetate/ hexanes) to give 0.63 g (42%)
of 3-(3-bromo-4-methoxybenzylidene)-1,3-dihydroindol-2-one as
a yellow solid.

15 ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.5 (m, 1H, NH), 7.7 (m, 1H,
Ar-H), 7.6 (m, 1H, Ar-CH=C), 7.5 (m, 1H, Ar-H), 7.2 (m, 2H,
2xAr-H), 6.9 (m, 1H, Ar-H), 6.8 (m, 1H, Ar-H), 3.9 (s, 3H,
OCH$_3$). MS m/z 330.0 and 332.0.

[M]$^+$ and [M+2]$^+$.

20 Tetrakis(triphenylphosphine)palladium(0) (0.02 g, 0.02
mmol) was added to a solution of 3-(3-Bromo-4-methoxy-
benzylidene)-1,3-dihydroindol-2-one (0.2 g, 0.61 mmol) in
toluene (1 ml) and ethanol (2 ml), followed by addition of 2M
aqueous sodium carbonate (1.2 ml, 2.4 mmol). To this mixture

25 was added 3-acetamidophenylboronic acid (0.12 g, 0.67 mmol),
and the mixture was heated to 100°C in a sealed tube and held
there for 12 hours. The reaction was then poured into water
(50 ml) and extracted with ethyl acetate (2x100 ml). The
combined organic layers were washed with saturated aqueous

30 sodium bicarbonate (50 ml) and brine (50 ml). The organic
layer was dried over magnesium sulfate and concentrated.
Chromatography (silica, 30-40% ethyl acetate/hexanes) afforded
a waxy solid that was triturated with diethyl ether/hexanes

and then dried to afford 0.05 g (22%) of *N*-[2'-methoxy-5'-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-biphenyl-3-yl]-acetamide as a reddish orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.6 (m, 1H, NH), 9.88 (s, 1H, NHAc), 7.7 (m, 1H, Ar-H), 7.5 (m, 3H, 2xAr-H and Ar-CH=C), 7.2 (m, 4H, Ar-H), 7.0 (m, 2H, Ar-H), 6.8 (m, 2H, Ar-H), 3.9 (two s, 3H, OCH$_3$), 2.02 (2, 3H, COCH$_3$).

MS m/z 385.2 [M+1]$^+$.

Example 30:   5-Fluoro-3-(5-isopropyl-4-methoxy-2-methyl-benzylidene)-1,3-dihydroindol-2-one

5-Isopropyl-4-methoxy-2-methylbenzaldehyde was condensed with 5-fluoro-2-oxindole to give 0.25 g of 5-fluoro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.54 (s, 1H, NH), 7.72 (s, 1H), 7.47 (s, 1H), 7.1 (dd, 1H), 7.04 (ddd, 1H), 6.98 (s, 1H), 6.82-6.86 (m, 1H), 3.86 (s, 3H, OCH$_3$), 3.25-3.28 (m, 1H, (CH$_3$)$_2$CH), 2.32 (s, 3H, CH$_3$), 1.14 (d, 6H, (CH$_3$)$_2$CH).

EI MS m/z 325 [M]$^+$.

Example 31:   *N*-[3-(4-Methoxy-3-thiophen-2-ylbenzylidene)-2-oxo-2,3-dihydro-1*H*-indol-6-yl]-acetamide

Tetrakis(triphenylphosphine)palladium(0) (0.24 g) was added to a solution of 4-methoxy-3-bromobenzaldehyde (1.5 g) in toluene (15 ml) and ethanol (15 ml), followed by addition of 2M aqueous sodium carbonate (14 ml). To this mixture was added thiophene-2-boronic acid (0.98 g), and the mixture was heated to reflux. After 3 hours, the reaction was partitioned between water (100 ml) and ethyl acetate (250 ml). The organic layer was washed with saturated aqueous sodium bicarbonate (75 ml) and brine (75 ml), dried over magnesium sulfate and concentrated. Chromatography (silica, 20% ethyl acetate/hexanes) afforded 1.3 g (87%) of 3-(2-thiophene)-4-methoxybenzaldehyde as a yellow oil.

¹HNMR (360 MHz, DMSO-d6) δ: 9.92 (s, 1H, CHO), 8.2 (d, 1H, J = 3Hz, 1xAr-H), 7.8 (dd, 1H, J = 10 and 10Hz, SCHCHCH), 7.65 (m, 1H, Ar-H), 7.60 (dd, 1H, J = 2 and 6Hz, Ar-H), 7.32 (d, 1H, J = 10Hz, SCHCHCH), 7.13 (d, 1H, J = 10 and 6Hz, SCHCHCH),

5   3.99 (s, 3H, OCH₃).

A mixture of 3-(2-thiophene)-4-methoxybenzaldehyde (0.25 g, 1.15 mmol), 6-acetamido-2-oxindole (0.22g, 1.15 mmol) and pyrrolidine (0.48 ml, 5.75 mmol) in ethanol (4 ml) was held in a sealed tube at 100°C for 12 hours. The reaction was cooled

10   and the precipitate which formed was removed by filtration, washed with ethanol and then hexanes and dried to afford 0.15 g (33%) of N-[3-(4-methoxy-3-thiophen-2-ylbenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide as a yellow solid.

¹HNMR (360 MHz, DMSO-d6) δ: 10.49 (s, 1H, NH), 9.95 (s,

15   1H, NHAc), 8.99 (d, J = 2Hz, 1H, Ar-H), 8.28 (dd, J = 2 and 9Hz, 1H, Ar-H), 7.6 (m, 2H, 2xAr-H), 7.5 (m, 2H, Ar-H and Ar-CH=C), 7.38 (d, 1H, J =1Hz, Ar-H), 7.22 (d, 1H, J = 9Hz, Ar-H), 7.14 (dd, 1H, J = 4 and 5Hz, Ar-H), 7.05 (dd, 1H, J =2 and 9Hz, Ar-H), 3.97 (s, 3H, OCH₃), 2.04 (s, 3H, NHCOCH₃).

20   MS m/z 391.3 [M]⁺ and [M+2]⁺.

Example 32:   6-Amino-3-(3,5-diisopropyl-4-methoxy-benzylidene)-1,3-dihydroindol-2-one

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 6-amino-2-oxindole to give 0.3 g of 6-amino-3-(3,5-

25   diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 33:   N-[3-(2,2-Dimethylchroman-6-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide

A mixture of 6-acetamido-2-oxindole (285 mg), 2,2-

30   dimethylchroman-6-carboxaldehyde (285 mg) and piperidine (0.2 ml) was stirred at 90°C overnight. The reaction mixture was cooled and diluted with 20 ml of water. The sticky solid which formed was filtered and chromatographed (2% ethanol in ethyl

acetate) to give 240 mg (44%) of N-[3-(2,2-dimethylchroman-6-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide as an orange red solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.44 (s, 1H, NH), 10.0 (s, 1H, NH), 7.6, 7.46, 7.42, 7.37, 6.93, 6.82 (m, 7H, 6 Ar-H and 1 vinyl-H), 2.8 (t, J = 6Hz, 2H, CH$_2$), 2.03 (s, 3H, CH$_3$), 1.83 (t, J = 6Hz, 2H, CH$_2$), 1.32 (s, 6H, 2x CH$_3$).

MS m/z 363.1 [M+1]$^+$.

Example 34: **5-Bromo-3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one**

2-Oxindole (1.3 g) in 20 ml of acetonitrile was cooled to -10°C and 2.0 g of N-bromosuccinimide was slowly added with stirring. The reaction was stirred for 1 hour at -10°C and then 2 hours at 0°C. The precipitate which formed was collected, washed with water and dried to give 1.9 g (90% yield) of 5-bromo-2-oxindole.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.44 (s, br, 1H, NH-1), 7.32-7.36 (m, 2H), 6.76 (d, J = 8.5 Hz, 1H, H-7), 3.5 (s, 2H, CH$_2$).

MS m/z 212.1 and 214.1, [M]$^+$ and [M+2]$^+$.

2,2-Dimethyl-6-formylchromane (commercially available) was condensed with 5-bromo-2-oxindole to give 0.3 g of 5-bromo-3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one as a yellow-orange solid.

Isomer 1 (67%): ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.65 (s, br, 1H, NH), 7.73 (d, 1H), 7.6 (s, 1H, vinyl), 7.5 (s, br, 1H), 7,47 (dd, 1H), 7.37 (dd, 1H), 6.86 (d, 1H), 6.82 (d, 1H), 2.77-2.81 (m, 4H, 2 x CH$_2$), 1.81-1.84 (m, 4H, 2 x CH$_2$), 1.32 (s, 6H, 2 x CH$_3$).

Isomer 2 (33%): ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.63 (s, br, 1H, NH), 8.32 (dd, 1H), 8.27 (s, br, 1H), 7.89 (d, 1H), 7,81 (s, 1H, vinyl), 7.3 (dd, 1H), 6.79 (d, 1H), 6.76 (d, 1H), 2.77-2.81 (m, 4H, 2 x CH$_2$), 1.81-1.84 (m, 4H, 2 x CH$_2$), 1.31 (s, 6H, 2 x CH$_3$).

MS APCI m/z 384.1 and 385.9, [M]$^+$ and [M+2]$^+$.

Example 35:   3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-1,3-dihydroindol-2-one

Tetrakis(triphenylphosphine)palladium(0) (0.02 g, 0.02
5   mmol) was added to a solution of 3-(3-bromo-4-methoxy-
benzylidene)-1,3-dihydroindol-2-one (0.2 g, 0.61 mmol) in
toluene (1 ml) and ethanol (1 ml), followed by addition of 2M
aqueous sodium carbonate (1.2 ml, 2.4 mmol). To this mixture
was added thiophene-3-boronic acid (0.09 g, 0.67 mmol) and the
10   mixture was held at 100°C in a sealed tube for 12 hours. The
reaction was then poured into water (50 ml) and extracted with
ethyl acetate (2x100 ml). The combined organic layers were
washed with saturated aqueous sodium bicarbonate (50 ml) and
brine (50 ml), dried over magnesium sulfate and concentrated.
15   Chromatography (silica, methylene chloride then 30-40% ethyl
acetate/hexanes) afforded 0.1 g (50%) of 3-(4-methoxy-3-
thiophen-3-ylbenzylidene)-1,3-dihydroindol-2-one as a yellow
solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.54 (s, 1H, NH), 7.8 (m, 2H,
20   2xAr-H), 7.6 (m, 5H, 4xAr-H and Ar-CH=C), 7.2 (m, 2H, Ar-H),
6.8 (m, 2H, Ar-H), 3.92 (s, 3H, OCH$_3$).

MS m/z 334.3 [M+1]$^+$.

Example 36:   5-Bromo-3-(5-isopropyl-4-methoxy-2-methyl-benzylidene)-1,3-dihydroindol-2-one

25   5-Isopropyl-4-methoxy-2-methylbenzaldehyde was condensed
with 5-bromo-2-oxindole to give 0.3 g of 5-bromo-3-(5-
isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-
one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.5 (s, 1H, CONH), 7.7, 7.6, 7.5, 7.4,
30   7.0, 6.8 (m, 6H, aromatic, =CH-), 3.9 (s, 3H, OCH$_3$), 3.3 (m,
1H, CH), 2.3 (s, 3H, CH$_3$), 1.2 (d, 6H, 2xCH$_3$).

Example 37:   5-Fluoro-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

4-Methoxy-3-phenylbenzaldehyde was condensed with 5-fluoro-2-oxindole to give 0.3 g of 5-fluoro-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 38:   3-(3-Isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

Diethyl oxalate (30 ml) in 20 ml of dry ether was added with stirring to 19 g of potassium ethoxide suspended in 50 ml of dry ether. The mixture was cooled in an ice bath and 20 ml of 3-nitro-o-xylene in 20 ml of dry ether was slowly added. The thick dark red mixture which formed was refluxed for 0.5 hour, concentrated to a dark red solid, and treated with 10 % sodium hydroxide until almost all of the solid dissolved. The dark red mixture was treated with 30% hydrogen peroxide until the red color changed to yellow. The mixture was then treated alternately with 10% sodium hydroxide and 30% hydrogen peroxide until the dark red color no longer formed on addition of sodium hydroxide. The remaining solid was filtered and the filtrate acidified with 6 N hydrochloric acid. The resulting precipitate was collected by vacuum filtration, washed with water, and dried under vacuum to give 9.8 g (45% yield) of 1-methyl-6-nitrophenylacetic acid as an off-white solid. The solid was hydrogenated in methanol over 10% palladium on carbon to give 9.04 g of 4-methyl-2-oxindole as a white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.27 (s, br, 1H, NH), 7.06 (t, J = 8Hz, 1H, H-6), 6.74 (d, J = 8Hz, H-5), 6.63 (d, J = 8Hz, 1H, H-7), 3.36 (s, 2H, CH_2), 2.18 (s, 3H, CH_3).

3-Isopropyl-4-methoxybenzaldehyde was condensed with 4-methyl-2-oxindole to give 0.25 g of 3-(3-isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (d_6-DMSO) δ: 10.4(s, 1H, NH), 8.2, 8.1, 7.6, 7.2,

7.1, 6.8, 6.7 (m, 7H, aromatic, =CH-), 3.9 (s, 3H, OCH₃), 3.2
(m, 1H, CH), 2.6 (m, 1H, CH), 1.2 (d, 6H, 2xCH₃).

**Example 39:   3-(4,5-Dimethoxy-2-thiophen-2-ylbenzylidene)-
1,3-dihydroindol-2-one**

5   A mixture of 6-bromoveratraldehyde (1 g, 4.08 mmol),
oxindole (0.54 g, 4.08 mmol) and pyrrolidine (1.7 ml, 20.4
mmol) in dimethylformamide (6 ml) was held in a sealed tube at
100°C for 12 hours. The reaction mixture was cooled and added
to 1N hydrochloric acid (100 ml). The precipitate which formed
10   was filtered and washed with water. The precipitate was then
dissolved in ethyl acetate (200 ml), the solution washed with
brine, dried over magnesium sulfate and concentrated. The
solid obtained was chromatographed (silica, 30-4-% ethyl
acetate/ hexanes) to give 1.2 g (81%) of 3-(3-bromo-4,5-
15   dimethoxybenzylidene)-1,3-dihydroindol-2-one as a yellow
solid.

        ¹HNMR (360 MHz, DMSO-d6) δ: 10.5 (s, 1H, NH), 7.4 (s, 1H,
Ar-CH=C), 7.3 (m, 2H, 2xAr-H), 7.2 m, 1H, Ar-H), 6.8 (m, 2H,
2xAr-H), 3.8 (s, 3H, OCH₃), 3.7 (s, 3H, OCH₃).

20   MS m/z 360.5 and 362.5, [M]⁺ and [M+2]⁺.

        Tetrakis(triphenylphosphine)palladium(0) (0.03 g, 0.03
mmol) was added to a solution of 3-(3-bromo-4,5-
dimethoxybenzylidene)-1,3-dihydroindol-2-one (0.32 g, 0.89
mmol) in toluene (2 ml) and ethanol (2 ml), followed by
25   additiona of 2M aqueous sodium carbonate (1.8 ml, 3.6 mmol).
To this mixture was added thiophene-2-boronic acid (0.13 g,
0.98 mmol), and the mixture was held at 100°C in a sealed tube
for 12 hours. The reaction mixture was poured into water (50
ml) and extracted with ethyl acetate (2x100 ml). The combined
30   organic layers were washed with saturated aqueous sodium
bicarbonate (50 ml) and brine (50 ml), dried over magnesium
sulfate and concentrated. Chromatography (silica, 30-40%
ethyl acetate/hexanes) afforded an orange solid that was

tr* triturated with methylene chloride and dried to afford 0.19 g
(59%) Of 3-(4,5-dimethoxy-2-thiophen-2-ylbenzylidene)-1,3-
dihydroindol-2-one as an orange solid.

¹HNMR (360 MHz, DMSO-d6) δ: 10.5 (m, 1H, NH), 7.6 (m, 1H,
5   Ar-H), 7.5 (m, 2H, Ar-H), 7.3 (m, 1H, Ar-CH=C), 7.2 (m, 4H,
Ar-H), 6.8 (m, 2H, Ar-H), 6.8 (m, 2H, Ar-H), 3.9 (two s, 3H,
OCH₃), 3.7 (two s, 3H, COCH₃).

MS m/z 364.0 [M+1]⁺.

10 **Example 40:   N-{3-[4-(2-Morpholin-4-ylethoxy)-3-thiophen-2-
ylbenzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide**

Triphenylphosphine (6.85 g) was added to a solution of 3-
bromo-4-hydroxybenzaldehye (5 g) in tetrahydrofuran (40 ml),
followed by addition of 2-hydroxyethylmorpholine (3.01 ml) and
15 then the dropwise addition of diethylazodicarboxylate (4.11
ml). After 12 hours, the reaction was concentrated under
reduced pressure, poured into 2N hydrochloric acid (200 ml)
and extracted with ethyl acetate (2x 150 ml). The aqueous
layer was basified to pH 9 with solid sodium bicarbonate and
20 extracted with ethyl acetate (2 x 150 ml). The organic layers
were dried over magnesium sulfate and concentrated to afford
4.4 g (56%) of 4-morpholinoethoxy-3-bromobenzaldehyde as a
reddish oil.

¹HNMR (360 MHz, DMSO-d6) δ: 9.84 (s, 1H, NH), 8.07 (d, 1H,
25 J = 2Hz, Ar-H), 7.90 (dd, 1H, J = 2 and 8Hz, Ar-H), 7.32 (d,
1H, J = 8Hz, Ar-H), 4.29 (t, 2H, J = 6Hz, Ar-OCH₂), 3.56 (m,
4H, OCH₂CH₂N), 2.76 (t, 2H, J = 6Hz, ArOCH₂-CH₂N), 2.50 (m, 4H,
NCH₂CH₂O).

Tetrakis(triphenylphosphine)palladium(0) (0.23) was added
30 to a solution of 4-morpholinoethoxy-3-bromobenzaldehyde (2.13
g) in toluene (10 ml) and ethanol (10 ml), followed by addition
of 2M aqueous sodium carbonate (13 ml). To this mixture was
added thiophene-2-boronic acid (1.13 g), and the mixture was

refluxed for 12 hours. The reaction mixture was then poured into water (100 ml) and extracted into ethyl acetate (3 x 100 ml). The product was extracted into 1N hydrochloric acid (2 x 100 ml), the aqueous layers basified to pH 9 with solid sodium bicarbonate and extracted with ethyl acetate (2 x 100ml). The organic layers were washed with brine (75 ml), dried over magnesium sulfate and concentrated to afford 1.7 g (75%) of 3-(2-thiophene)-4-morpholinoethoxybenzaldehyde as a greenish/yellow oil.

^{1}HNMR (360 MHz, DMSO-d6) δ: 9.93 (s, 1H, CHO), 8.2 (d, 1H, J = 2Hz, Ar-H), 7.82 (dd, 1H, J = 2 and 8Hz, Ar-H), 7.76 (dd, 1H, J = 1 and 4Hz, Ar-H), 7.59 (dd, 1H, J = 1 and 5Hz, Ar-H), 7.35 (d, 1H, J = 9Hz, Ar-H), 7.14 (dd, 1H, J = 4 and 5Hz, Ar-H), 4.34 (t, J = 6Hz, 2H, O-CH_2CH_2), 3.56 (m, 4H, 2x ringO-CH_2CH_2), 2.82 (t, J = 6Hz, 2H, N-CH_2CH_2), 2.5 (m on DMSO, 4H, 2x ringN-CH_2CH_2).

MS m/z 318.2 [M+1]$^+$.

A mixture of 3(2-thiophene)-4-morpholinoethoxy-benzaldehyde (1.74 g), 6-acetamido-2-oxindole(1g) and piperidine (2.6 ml) in ethanol (11 ml) was held in sealed tube at 100°C for 12 hours. The reaction was cooled and added to diethyl ether and the ether decanted. The oily residue was dissolved in methylene chloride (10 ml) and precipitated in ether (200 ml) and hexanes (100 ml). The precipitate was removed by filtration, washed with ether and then hexanes and dried to afford a brownish solid. Chromatography (silica, 5% methanol/dichloromethane) afforded N-{3-[4-(2-morpholin-4-ylethoxy)-3-thiophen-2-ylbenzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide as a dark yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.49 (s, 1H, NH), 10.01 (s, 1H, NHAc), 8.05 (d, J = 2Hz, 1H, Ar-H), 7.72 (m, 1H, Ar-H), 7.65 (m, 1H, Ar-H), 7.48 (m, 2H, 2xAr-H), 7.47 (s, 1H, Ar-CH=C), 7.45 (d, 1H, J =2Hz, Ar-H), 7.29 (d, 1H, J = 9Hz, Ar-

H), 7.13 (m, 1H, Ar-H), 6.90 (dd, 1H, J =2 and 9Hz, Ar-H),
4.32 (m, 2H, ArOCH$_2$CH$_2$N), 3.58 (m, 4H, 2xOCH$_2$CH$_2$N), 2.84 (m, 2H,
ArOCH$_2$CH$_2$N), 2.5 (m under DMSO, 4H, 2xOCH$_2$CH$_2$N), 2.03 (s, 3H,
NHCOCH$_3$).

5   MS m/z 490.0 [M+1]$^+$.

Example 41:   **3-(2,2-Dimethylchroman-6-ylmethylene)-4-methyl-1,3-dihydroindol-2-one**

         2,2-Dimethyl-6-formylchromane (commercially available)
was condensed with 4-methyl-2-oxindole to give 0.25 g of 3-
10 (2,2-dimethylchroman-6-ylmethylene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

         ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.45 (s, br, 1H, NH), 8.17
(dd, J = 2, 8HZ, 1H), 8.02 (d, J = 2Hz, 1H), 7.6 (s, 1H,
vinyl), 7.05 (t, J = 8Hz, 1H), 6.75 (t, 2H), 6.67 (d, J = 8Hz,
15 1H), 2.77 (t, J = 7Hz, 2H, CH$_2$), 2.55 (s, 3H, CH$_3$), 1.8 (t, J =
7Hz, 2H, CH$_2$), 1.31 (s, 6H, 2 x CH$_3$).

         MS APCI m/z 320.2 [M+1]$^+$.

Example 42:   **3-(2,3-Dihydrobenzofuran-5-ylmethylene)-5-fluoro-1,3-dihydroindol-2-one**

20   2,3-Dihydro-5-formylbenzofuran (commercially available)
was condensed with 5-fluoro-2-oxindole to give 0.25 g of 3-
(2,3-dihydrobenzofuran-5-ylmethylene)-5-fluoro-1,3-
dihydroindol-2-one as a yellow-orange solid.

Example 43:   **3-(3-Cyclohexyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one**

25   2-Cyclohexylphenol was methylated then formylated. The
aldehyde was condensed with 5-fluoro-2-oxindole to give 0.3 g
of 3-(3-cyclohexyl-4-methoxybenzylidene)-5-fluoro-1,3-
dihydroindol-2-one as a yellow-orange solid.

Example 44:   **5-Fluoro-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one**

30   3-Isopropyl-4-methoxybenzaldehyde was condensed with 5-
fluoro-2-oxindole to give 0.25 g of 5-fluoro-3-(3-isopropyl-4-

methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange
solid.

**Example 45:   3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-
1,3-dihydro-pyrrolo[2,3-b]pyridin-2-one**

5   Zinc dust (6.5 g) was added to a solution of 3,3-dibromo-
7-azaoxindole (2.9 g) in acetic acid (20 ml) and acetonitrile
(30 ml). The mixture was stirred for 2 hours at room
temperature. The solids were removed by filtration and the
solvent evaporated. The residue was treated with ethyl

10   acetate. The ethyl acetate solution containing insoluble
solid was passed through a short column of silica gel. The
collected ethyl acetate solution was evaporated and the
residue dried under vacuum to give 1.8 g (91% yield) of 7-aza-
2-oxindole acetic acid salt.

15   ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.90 (s, br, 1H, NH), 8.01,
7.52, 6.91 (m, 3H), 3.52 (s, 2H, CH$_2$).

       MS APCI 135 (M+1).

       5-Isopropyl-4-methoxy-2-methylbenzaldehyde condensed with
7-aza-2-oxindole to give 0.25 g of 3-(5-isopropyl-4-methoxy-2-

20   methylbenzylidene)-1,3-dihydro-pyrrolo[2,3-b]pyridin-2-one as
a yellow-orange solid.

       ^{1}HNMR (d$_6$-DMSO) δ: 10.5(s, 1H, CONH), 8.3-6.8(m, 6H,
aromatic, =CH-), 3.9(s, 3H, OCH$_3$), 3.3(m, 1H, CH), 2.3(s, 3H,
CH$_3$), 1.2(d, 6H, 2xCH$_3$).

25   **Example 46:   3-(3'-Ethoxy-6-methoxybiphenyl-3-ylmethylene)-
1,3-dihydroindol-2-one**

       Tetrakis(triphenylphosphine)palladium(0) (0.02 g, 0.02
mmol) was added to a solution of 3-(bromo-4-methoxy-
benzylidene)-1,3-dihydroindol-2-one (0.2 g, 0.61 mmol) in

30   toluene (1 ml) and ethanol (1 ml), followed by addition of 2M
aqueous sodium carbonate (1.2 ml, 2.4 mmol). To this mixture
was added 3-ethoxyphenylboronic acid (0.11 g, 0.67 mmol), and
the mixture was held at100°C in a sealed tube for 12 hours.

The reaction mixture was added to water (40 ml) and extracted with ethyl acetate (75 ml). The combined organic layers were washed with saturated aqueous sodium bicarbonate (50 ml) and brine (50 ml). The organic layer was dried over magnesium

5   sulfate and concentrated. The resulting solid was triturated with dichloromethane/ hexanes to afford 0.9 g (39%) of 3-(3'-ethoxy-6-methoxy-biphenyl-3-ylmethylene)-1,3-dihydroindol-2-one as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.57 (s, 1H, NH), 7.65 (m,
10   5H, 4xAr-H and Ar-CH=C), 7.3 (m, 3H, 4xAr-H), 7.15 (m, 2H, Ar-H), 6.9 (m, 2H, Ar-H), 4.05 (q, 2H, OCH$_2$CH$_3$), 3.85 (s, 3H, OCH$_3$), 1.35 (t, 3H, OCH$_2$CH$_3$).

MS m/z 372.5 [M+1]$^+$.

Example 47:   **3-(3-Cyclopentyl-4-methoxybenzylidene)-1,3-**
15   **dihydropyrrolo[2,3-b]pyridin-2-one**

3-Cyclopentyl-4-methoxybenzaldehyde was condensed with 7-aza-2-oxindole to give 0.25 g of 3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one as a yellow-orange solid.

20   ^{1}HNMR (d$_6$-DMSO) δ: 1.1 (s, 1H, CONH), 8.3-6.8 (m, 7H, aromatic, =CH-), 3.9(s, 3H, OCH$_3$), 3.2(m, 1H, CH), 1.8 (m, 8H, 4xCH$_2$).

Example 48:   **3-(3-Cyclopentyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one**

25   3-Cyclopentyl-4-methoxybenzaldehyde was condensed with 4-methyl-2-oxindole to give 0.25 g of 3-(3-cyclopentyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2- as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.4 (s, 1H, CONH), 8.3, 8.1, 7.6, 7.0,
30   7.0, 6.8, 6.6 (m, 7H, aromatic, =CH-), 3.9 (s, 3H, OCH$_3$), 3.2 (m, 1H, CH), 1.8 (m, 8H, 4xCH$_2$).

Example 49:   **3-(4,5,2'-Trimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one**

Tetrakis(triphenylphosphine)palladium(0) (0.02 g, 0.02 mmol) was added to a solution of 3-(3-bromo-4,5-dimethoxybenzylidene)-1,3-dihydroindol-2-one (0.20 g, 0.56 mmol) in toluene (1ml) and ethanol (1 ml), followed by

5   addition of 2M aqueous sodium carbonate (1.1 ml, 2.2 mmol). To this mixture was added 2-methoxyphenylboronic acid (0.09 g, 0.62 mmol), and the mixture was held at 100°C in a sealed tube for 12 hours. The reaction mixture was added to water (50 ml) and extracted with ethyl acetate (2x100 ml). The combined

10   organic layers were washed with saturated aqueous sodium bicarbonate (50 ml) and brine (50 ml). The organic layer was dried over magnesium sulfate and concentrated. Chromatography (silica, 30-40% ethyl acetate / hexanes) afforded 0.14 g (64%) of 3-(4,5,2'-trimethoxy-biphenyl-2-ylmethylene)-1,3-

15   dihydroindol-2-one as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.4 (m, 1H, NH), 7.5 (m, 1H, Ar-H), 7.3 (m, 2H, Ar-H and Ar-CH=C), 7.2 (m, 4H, Ar-H), 6.9 (m, 4H, Ar-H), 3.8 (two s, 3H, COCH$_3$), 3.7 (two s, 3H, OCH$_3$), 3.5 (two s, 3H, COCH$_3$).

20   MS m/z 388.2 [M+1]$^+$.

Example 50:   N-{3-[4-(2-Morpholin-4-ylethoxy)-3-thiophen-3-ylbenzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

Tetrakis(triphenylphosphine)palladium(0) (0.21 g) was added to a solution of 4-morpholinoethoxy-3-bromobenzaldehyde

25   (1.92 g) in toluene (10 ml) and ethanol (10 ml), followed by addition of 2M aqueous sodium carbonate (12 ml). To this mixture was added thiophene-3-boronic acid (1.02 g), and the mixture was heated to reflux. After 12 hours, the reaction mixture was added to water (100 ml) and extracted into ethyl

30   acetate (3 x 100 ml). The product was then extracted into 1N hydrochloric acid (2 x 100 ml), the aqueous layers basified to pH 9 with solid sodium bicarbonate and extracted with ethyl acetate (2 x 100 ml). The organic layers were washed with

brine (75 ml), dried over magnesium sulfate and concentrated
to afford 1.7 g (83%) of 3-(3-thiophene)-4- morpholinoethoxy
benzaldehyde a reddish oil.

¹HNMR (360 MHz, DMSO-d6) δ: 9.91 (s, 1H, CHO), 8.08 (m,
2H, Ar-H), 7.83 (dd, 1H, J = 2 and 9Hz, Ar-H), 7.76 (dd, 1H, J
= 1 and 4Hz, Ar-H), 7.59 (dd, 1H, J = 1 and 5Hz, Ar-H), 7.35
(d, 1H, J = 9Hz, Ar-H), 7.64 (dd, 1H, J = 1 and 5Hz, Ar-H),
7.58 (dd, 1H, J = 3 and 5Hz, Ar-H), 7.31 (d, 1H, J = 9Hz, Ar-
H), 4.27 (t, J = 5Hz, 2H, O-CH₂CH₂), 3.56 (m, 4H, 2x ringO-
CH₂CH₂), 2.75 (t, J = 6Hz, 2H, N-CH₂CH₂), 2.48 (m on DMSO, 4H,
2x ringN-CH₂CH₂).

MS m/z 318.2 [M+1]⁺.

A mixture of 3-(3-thiophene)-4-morpholinoethoxy-
benzaldehyde (1.74 g), 6-acetamido-2-oxindole (1 g) (prepared
as described in example 1) and piperidine (2.6 ml) in ethanol
(11 ml) was held in a sealed tube at 100°C for 12 hours. The
reaction was cooled and added to diethyl ether (200 ml). The
solvent was decanted, and the oily solid which remained was
dissolved in dichloromethane (15 ml) and precipitated in
diethyl ether (200 ml) and hexanes (200 ml), filtered, washed
with diethyl ether and then hexanes and dried to afford a
mustard yellow solid. Chromatography (silica, 5% methanol/
dichloromethane) afforded N-{3-[4-(2-morpholin-4-ylethoxy)-3-
thiophen-3-ylbenzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-
acetamide as a yellow foam.

¹HNMR (360 MHz, DMSO-d6) δ: 10.47 (s, 1H, NH), 10.00 (s,
1H, NHAc), 8.07 (m, 1H, Ar-H), 7.91 (d, J = 2Hz, 1H, Ar-H),
7.63 (m, 2H, 2xAr-H), 7.58 (m, 2H, 2xAr-H), 7.48 (s, 1H, Ar-
CH=C), 7.45 (d, 1H, J = 2Hz, Ar-H), 7.26 (d, 1H, J = 9Hz, Ar-
H), 6.90 (dd, 1H, J =2 and 9Hz, Ar-H), 4.26 (m, 2H,
ArOCH2CH2N), 3.58 (m, 4H, 2xOCH₂CH₂N), 2.76 (m, 2H,
ArOCH2CH2N), 2.48 (m under DMSO, 4H, 2xOCH₂CH₂N), 2.03 (s, 3H,
NHCOCH₃).

MS m/z 490.0 [M+1]⁺.

Example 51:   5-Chloro-3-(3-cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

2-Cyclohexylphenol was methylated and then formylated. The aldehyde was condensed with 5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(3-cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

¹HNMR (d₆-DMSO) δ: 10.6(s, 1H, CONH), 7.7-6.8(m, 7H, aromatic, =CH-), 3.9(s, 3H, OCH₃), 3.0 (m, 1H, CH), 1.8, 1.4 (m, 10H, 5xCH₂).

Example 52:   [3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-carbamic acid tert-butyl ester

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 6-tert-butyloxycarbonylamino-2-oxindole to give 0.35 g of [3-(3,5-diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-carbamic acid tert-butyl ester as a yellow-orange solid.

Example 53:   3-(3,5-Diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 4-methyl-2-oxindole to give 0.3 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

¹HNMR (d₆-DMSO) δ: 10.41 (s, 1H, CONH), 8.0 (s, 2H), 7.66 (s, 1H), 7.07 (t, 1H), 6.76 (d, 1H), 6.67 (d, 1H), 3.7 (s, 3H, OCH₃), 3.21-3.3 (m, 1H), 1.21 (d, 12H, 4 x CH₃).

Example 54:   5-Bromo-3-(3,5-diisopropyl-4-methoxy-benzylidene)-1,3-dihydroindol-2-one

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5-bromo-2-oxindole to give 0.3 g of 5-bromo-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 55:   N-{3-[3-tert-Butyl-4-(2-morpholin-4-ylethoxy)-
benzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

Triphenylphosphine (5.89 g, 22.44 mmol) was added to a
solution of 3-tert-butyl-4-hydroxybenzaldehyde (4 g, 22.44
5   mmol) in tetrahydrofuran (40 ml), followed by addition of 2-
hydroxyethylmorpholine (2.94 g, 22.44 mmol) and then the
dropwise addition of diethylazodicarboxylate (3.91 g, 22.44
mmol). The mixture was allowed to stir at room temperature for
3 days. The reaction was concentrated under reduced pressure
10   and partitioned between 2N hydrochloric acid (200 ml) and
ethyl acetate (150 ml). The aqueous layer was extracted with
ethyl acetate (2 x 150 ml), basified to pH 9 with solid sodium
bicarbonate, saturated with solid sodium chloride and
extracted with ethyl acetate (3 x 150 ml). The combined
15   organic layers were dried over magnesium sulfate and
concentrated to afford 2.8 g (44%) of 3-tert-butyl-4-(2-
morpholin-4-ylethoxy)benzaldehyde as a yellowish oil.

^{1}HNMR (360 MHz, DMSO-d6) δ: 9.8 (s, 1H, CHO), 7.75 (m,
2H, 2xAr-H), 7.16 (d, J = 10Hz, 1H, Ar-H), 4.19 (t, J = 7Hz,
20   2H, O-CH$_2$CH$_2$), 3.55 (m, 4H, 2x ringO-CH$_2$CH$_2$), 2.75 (t, J = 7Hz,
2H, N-CH$_2$CH$_2$), 2.4 (m on DMSO, 4H, 2x ringN-CH$_2$CH$_2$), 1.36 (s,
9H, C(CH$_3$)$_3$).

MS m/z 292.0 [M+1]$^+$.

A mixture of 3-tert-butyl-4-(2-morpholin-4-ylethoxy)-
25   benzaldehyde (0.25 g), 6-acetamido-2-oxindole (0.16g) and
piperidine (0.43 ml) in ethanol (3 ml) was held in a sealed
tube at 100°C for 12 hours. The reaction was cooled, added to
ethyl acetate (250 ml), washed with 1N hydrochloric acid (100
ml) and brine (100 ml), dried with magnesium sulfate and
30   concentrated. Some product went into the acid wash, so this
was basified with solid sodium bicarbonate to pH 9 and
extracted with ethyl acetate (2 x 150 ml). The organic layers
were dried over magnesium sulfate, combined with the oil from

the first crop of product and concentrated. Chromatography (silica, 40% ethyl acetate followed by 5-10% methanol / methylene chloride) afforded 100 mg (27%) of N-{3-[3-tert-butyl-4-(2-morpholin-4-ylethoxy)benzylidene]-2-oxo-2,3-
5   dihydro-1H-indol-6-yl}-acetamide as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.46 (s, 1H, NH), 9.99 (s, 1H, NHAc), 7.58 (m, 3H, Ar-H), 7.41 (m, 2H, Ar-H and Ar-CH=C), 7.12 (d, 1H, Ar-H), 6.92 (dd, 1H, J =2 and 8Hz, Ar-H), 4.18 (m, 2H, Ar-OCH$_2$), 3.57 (m, 4H, OCH$_2$CH$_2$N), 2.77 (m, 2H, ArOCH$_2$-
10   CH$_2$N), 2.49 (m, 4H, NCH$_2$CH$_2$O), 2.02 (s, 3H, NHCOCH$_3$), 1.38 (s, 9H, Ar-tBu).

MS m/z 464.0 [M+1]$^+$.

Example 56:   <u>3-(4-Methoxy-3,5-dimethylbenzylidene)-1,3-
dihydropyrrolo[2,3-b]pyridin-2-one</u>

15   2,6-Dimethylphenol was methylated and then formylated.

^{1}HNMR (d$_6$-DMSO) δ: 10.1 (s, 1H, CHO), 6.9 (s, 2H, aromatic), 3.9 (s, 3H, OCH$_3$), 2.6 (s, 6H, 2xCH$_3$).

The aldehyde was condensed with 7-aza-2-oxindole to give 0.2 g of 3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-
20   dihydropyrrolo[2,3-b]pyridin-2-one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 1.5(s, 1H, CONH), 8.2-6.8(m, 6H, aromatic, =CH-), 3.8(s, 3H, OCH$_3$), 2.5(s, 6H, 2xCH$_3$), 2.4(s, 3H, CH$_3$).

25   **Example 57:**   <u>5-Bromo-3-[3,5-diisopropyl-4-(2-morpholin-4-
ylethoxy)benzylidene]-1,3-dihydroindol-2-one</u>

A mixture of 3,5-diisopropyl-4-(2-morpholin-4-ylethoxy)-benzaldehyde (0.4 g), 5-bromo-2-oxindole (0.27g) and pyrrolidine (0.5 ml) in ethanol (2 ml) was held in a sealed
30   tube at 100°C for 12 hours. The mixture was then added to 1N hydrochloric acid (100 ml) and the solids removed by filtration and washed with more water (50 ml). The solids were then dissolved in ethyl acetate (200 ml), the solution

washed with 1N hydrochloric acid (75 ml) and brine (75 ml),
dried with magnesium sulfate and concentrated. The crude
solid was chromatographed (silica, 4:4:1 dichloromethane:
hexanes: methanol) to give 80 mg (13%) of 5-bromo-3-[3,5-
5   diisopropyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-1,3-
dihydroindol-2-one as a reddish-brown solid.

       ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.54 (s, 1H, NH), 9.43 (d, J
= 10Hz, 1H, Ar-H), 7.58 (d, J = 9Hz, 1H, Ar-H), 7.46 (d, J =
5Hz, 2H, Ar-H), 7.3 (m, 1H, Ar-H), 7.19 (m, J = 9 and 10Hz,
10   1H, Ar-H), 6.99 (m, J = 9 and 9Hz, 1H, Ar-H), 6.83 (m, J = 9
and 1Hz, 1H, Ar-H), 3.61 (dd, J = 9 and 22Hz 1H, 1xCH$_2$CH), 3.43
(m, 1H, CHCH$_3$), 2.86 (dd, J = 4 and 22Hz, 1H, 1xCH$_2$CH), 1.35
(d, J = 9 Hz, 3H, CH$_3$).

       MS m/z 262.0 [M+1]$^+$.

15   **Example 58:   3-(3'-Ethoxy-4,5-dimethoxybiphenyl-2-
ylmethylene)-1,3-dihydroindol-2-one**

       Tetrakis(triphenylphosphine)palladium(0) (0.02 g, 0.02
mmol) was added to a solution of 3-(3-bromo-4,5-
dimethoxybenzylidene)-1,3-dihydroindol-2-one (0.2 g, 0.56
20   mmol) in toluene (1 ml) and ethanol (1 ml), followed by
addition of 2M aqueous sodium carbonate (1 ml, 2mmol). To
this mixture was added 3-ethoxyphenylboronic acid (0.1 g, 0.62
mmol) and the mixture was held at 100°C in a sealed tube for 12
hours. The reaction mixture was then added to water (40 ml)
25   and extracted with ethyl acetate (2 x 75 ml). The combined
organic layers were washed with saturated aqueous sodium
bicarbonate (50 ml) and brine (50 ml), dried over magnesium
sulfate and concentrated. The resulting solid was triturated
with methylene chloride / hexanes to afford 0.14 g (64%) of 3-
30   (3'-ethoxy-4,5-dimethoxybiphenyl-2-ylmethylene)-1,3-
dihydroindol-2-one as a yellow/orange solid.

       ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.5 (s, 1H, NH), 7.6 (d, J =
9Hz, 1H, Ar-H), 7.2 (m, 4H, 3xAr-H and Ar-CH=C), 7.08 (s, 2H,

Ar-H), 6.8 (m, 5H, 5xAr-H), 4.0 (q, J = 8Hz, 2H, OCH_2CH_3),

3.89 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 1.29 (t, J = 8Hz, 3H,

OCH_2CH_3).

MS m/z 402.5 $[M+1]^+$.

5   **Example 59:**   **5-chloro-3-(4-Methoxy-3-thiophen-2-ylbenzylidene)-
1,3-dihydroindol-2-one**

A mixture of 3-(2-thiophene)-4-methoxybenzaldehyde (0.21

g), 5-chloro-2-oxindole (0.16g) and pyrrolidine (0.4 ml) in

ethanol (1 ml) was placed in a sealed tube and heated to 100°C.

10   A precipitate formed within 10 minutes so the reaction was

diluted with another 10 ml of ethanol and heating resumed.

After 4 hours, the reaction was cooled and the solid which

formed was removed by filtration and washed with ethanol and

then hexanes to afford 0.21 g (60%) of 5-chloro-3-(4-methoxy-

15   3-thiophen-2-ylbenzylidene) -1,3-dihydroindol-2-one as a

yellow-orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.7 (s, 1H, NH), 7.8-9.1 (m,

3H, Ar-H), 7.6 (m, 3H, 2xAr-H and Ar-CH=C), 7.2 (m, 3H, Ar-H),

6.9 (m, 1H, Ar-H), 3.9 (m, 3H, OCH_3).

20   MS m/z 368.1 and 369.9 $[M]^+$ and $[M+2]^+$.

Example 60:   **5-Chloro-3-4-methoxy-3-pryidin-3-
ylbenzylidene)-1,3-dihydroindol-2-one**

Tetrakis(triphenylphosphine)palladium(0) (0.2 g) was

added to a solution of 3-bromo-4-methoxybenzldehyde (1.25 g)

25   in toluene (10 ml) and ethanol (10 ml), followed by addition

of 2M aqueous sodium carbonate (11 ml). To this mixture was

added pyridine-3-boronic acid propane diol (1 g), and the

mixture was heated to reflux. After 2 hours, the reaction was

added to water (75 ml) and extracted with ethyl acetate (2x 75

30   ml). The combined organic layers were washed with saturated

aqueous sodium bicarbonate (100 ml) and brine (100 ml), dried

over magnesium sulfate and concentrated. Chromatography

(silica, 20-50% ethyl acetate/hexanes) afforded 1.08 g (87%)

of 3-(3-pyridyl)-4-methoxybenzaldehyde as a white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 9.93 (s, 1H, CHO), 8.7 (d, 1H, J = 3Hz, Ar-H), 8.56 (dd, 1H, J = 1 and 6Hz, Ar-H), 7.9 (m, 3H, 3xAr-H), 7.46 (dd, 1H, J = 6 and 9Hz, Ar-H), 7.35 (d, 1H, 5 J = 10Hz, Ar-H), 3.89 (s, 3H, OCH$_3$).

A mixture of 3-(3-pyridyl)-4-methoxybenzaldehyde (0.25 g), 5-chloro-2-oxindole (0.2g) and pyrrolidine (0.5 ml) in ethanol (2 ml) was placed in a sealed tube and heated to 100°C. A precipitate formed within 10 minutes so the reaction was 10 diluted with another 10 ml of ethanol and heating resumed. After 4 hours, the reaction was cooled and the solid was removed by filtration and washed with ethanol and then hexanes to afford 0.27 g (61%) of 5-chloro-3-(3-(3-pyridyl)-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange 15 solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 0.67 (s, 1H, NH), 8.6 (m, 3H, 2xAr-H and Ar-CH=C), 7.8 (m, 4H, Ar-H), 7.4 (m, 1H, Ar-H), 7.35 (m, 2H, Ar-H), 6.85 (m, 1H, Ar-H), 3.88 (s, 3H, OCH$_3$).

MS m/z 363.0 and 365.1, [M]$^+$ and [M+2]$^+$.

20 **Example 61:   5-Chloro-3-(4,5-3′-Trimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one**

Tetrakis(triphenylphosphine)palladium(0) (0.36 g) was added to a solution of 4,5-dimethoxy-2-bromobenzaldehyde (2.5 g) in toluene (20 ml) and ethanol (20 ml), followed by 25 addition of 2M aqueous sodium carbonate (20 ml). To this mixture was added 3-methoxyphenylboronic acid (1.7 g), and the mixture was heated to reflux. After 1 hour, the reaction was partitioned between water (150 ml) and ethyl acetate (300 ml). The organic layer was washed with saturated aqueous sodium 30 bicarbonate (150 ml) and brine (150 ml), dried over magnesium sulfate and concentrated. Chromatography (silica, 20-30-40% ethyl acetate / hexanes) afforded 2.7 g (98%) of 2-(3-methoxyphenyl)-4,5-dimethoxybenzaldehyde as a yellow oil which

solidified upon standing.

^{1}HNMR (360 MHz, DMSO-d6) δ: 9.71 (s, 1H, CHO), 7.38 (m, 2H, 2xAr-H), 7.0 (m, 4H, 4xAr-H), 3.9 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$), 3.8 (s, 3H, OCH$_3$).

5   MS m/z 273.5 [M+1]$^+$.

A mixture of 2-(3-methoxyphenyl)-4,5-dimethoxy-benzaldehyde (0.25 g), 5-chloro-2-oxindole (0.15g) and pyrrolidine (0.4 ml) in ethanol (1 ml) was placed in a sealed tube and heated to 100°C. A precipitate formed within 10

10   minutes so the reaction was diluted with another 10 ml of ethanol and heating resumed. After 2.5 hours, the reaction was cooled, the solid was removed by filtration and washed with ethanol and then hexanes to afford 0.36 g (92%) of 3-(4,5-3'-trimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one as a

15   yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.67 (s, 1H, NH), 7.6 (d, J = 2.5Hz, 1H, Ar-H), 7.3 (m, 4H, Ar-H), 7.12 (s, 1H, Ar-CH=C), 6.9 (m, 4H, Ar-H), 3.9 (s, 3H, OCH$_3$), 3.79 (s, 3H, OCH$_3$), 3.75 (s, 3H, OCH$_3$).

20   MS m/z 422.0 and 424.1 [M]$^+$ and [M+2]$^+$.

Example 62:   3-(4,5-Dimethoxy-2-naphthalen-2-ylbenzylidene)-1,3-dihydroindol-2-one

Tetrakis(triphenylphosphine)palladium(0) (0.02 g) was added to a solution of 3-(3-bromo-4,5-dimethoxybenzylidene)-

25   1,3-dihydroindol-2-one (0.20 g) in toluene (1 ml) and ethanol (1 ml) followed by addition of 2M aqueous sodium carbonate (1.1 ml). To this mixture was added naphthalene-2-boronic acid (0.11 g), and the mixture was held at 100°C in a sealed tube for 12 hours. The precipitate which formed was filtered,

30   washed with ethanol and then dissolved in 20% methanol / toluene, filtered and concentrated. The resulting solid was triturated with hexanes and dried to afford 0.07 g (29%) of 3-(4,5-dimethoxy-2-naphthalen-2-ylbenzylidene)-1,3-dihydroindol-

2-one as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.5 (m, 1H, NH), 7.9 (m, 4H, Ar-H), 7.7 (m, 1H, Ar-H), 7.5 (m, 3H, Ar-H), 7.4 (s, 1H, Ar-CH=C), 7.2 (m, 3H, Ar-H), 6.9 (m, 2H, Ar-H), 3.9 (two s, 3H, COCH$_3$), 3.8 (two s, 3H, OCH$_3$).

MS m/z 408.0 [M+1]$^+$.

Example 63: **N-[3-(3'-Acetylamino-6-methoxybiphenyl-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide**

3-Bromo-4-methoxybenzaldehyde was condensed with 6-acetamido-2-oxindole to give N-[3-(3-bromo-4-methoxy-benzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide.

Tetrakis(triphenylphosphine)palladium(0) (0.01 g) was added to a solution of N-[3-(3-bromo-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide (0.14 g) in toluene (1 ml) and ethanol (1 ml) followed by addition of 2M aqueous sodium carbonate (0.72 ml). To this mixture was 3-acetylamino-phenylboronic acid (0.07 g), and the mixture was held at 100° C in a sealed tube for 12 hours. The reaction mixture was added to water (50 ml) and extracted with ethyl acetate (2x100 ml). The combined organic layers were washed with saturated aqueous sodium bicarbonate (50 ml) and brine (50 ml), dried over magnesium sulfate and concentrated. Chromatography (silica, dichloromethane then 5-10% methanol/ dichloromethane) afforded 0.07 g (43%) of N-[3-(3'-acetylamino-6-methoxybiphenyl-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide as a yellow solid. About 26% isomer by ^{1}HNMR.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.49 (s, 1H, NH), 10.42 (s, 1H, NHAc), 9.95 (s, 1H, NHAc), 7.76 (m, 2H, 2xAr-H), 7.58 (m, 3H, 3xAr-H), 7.4 (m, 2H, Ar-H and Ar-CH=C), 7.3 (m, 2H, Ar-H), 7.18 (m, 1H, Ar-H), 6.9 (dd, 2H, J = 2 and 8.5Hz, Ar-H), 3.84 (s, 3H, OCH$_3$), 2.05 (s, 3H, NHCOCH$_3$), 2.03 (s, 3H, NHCOCH$_3$).

MS m/z 442.2 [M+1]$^+$.

Example 64: **6-Methoxy-3-(4-methoxy-3-thiophen-3-**

ylbenzylidene)-1,3-dihydroindol-2-one

A mixture of 3(3-thiophene)-4-methoxybenzaldehyde (0.53 g, 2.45 mmol), 6-methoxy-2-oxindole (0.4g, 2.45 mmol) and piperidine (1.2 ml, 12.25 mmol) in 5 ml of ethanol was held in

5   a sealed tube at 100°C for 12 hours. The reaction was cooled and added to diethyl ether (150 ml) and hexanes (150 ml). The precipitate which formed was removed by filtration, washed with diethyl ether and then hexanes and dried to afford 0.57 g (64%) of 6-methoxy-3-(4-methoxy-3-thiophen-3-ylbenzylidene)-

10 1,3-dihydroindol-2-one as a mustard yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.45 (s, 1H, NH), 8.78 (d, J = 2Hz, 1H, Ar-H), 8.30 (dd, J = 2 and 9Hz, 1H, Ar-H), 7.8 (dd, 1H, J = 1.5 and 3Hz, Ar-H), 7.5 (m, 3H, 2xAr-H and Ar-CH=C), 7.18 (d, J = 9Hz, 1H, Ar-H), 6.55 (dd, 1H, J = 2 and 8.5Hz,

15 Ar-H), 6.39 (d, 1H, J = 2Hz, Ar-H), 3.91 (s, 3H, OCH$_3$), 3.76 (s, 3H, OCH$_3$), 2.04 (s, 3H, NHCOCH$_3$).

MS m/z 364.1 [M+1]$^+$.

Example 65:   3-(6-Methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

20   4-Methoxy-3-phenylbenzaldehyde was condensed with 2-oxindole to give 0.3 g of 3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.5(s, 1H, CONH), 8.5, 8.5, 7.8, 7.7, 7.5, 7.5, 7.4, 7.4, 7.3, 7.2, 7.2, 7.0, 6.8(m, 13H, aromatic,

25 =CH-), 3.9(s, 3H, OCH$_3$).

Example 66:   3-(2,3-Dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one

2,3-Dihydro-5-formylbenzofuran (commercially available) was condensed with 2-oxindole to give 0.25 g of 3-(2,3-

30 dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.4 (s, 1H, CONH), 8.6, 8.2, 7.7, 7.6, 7.2, 7.0, 6.9, 6.8 (m, 8H, aromatic, =CH-), 4.6(t, 2H, OCH$_2$),

3.2 (t, 2H, OCH$_2$).

Example 67: <u>5-Chloro-3-(6-methoxybiphenyl-3-ylmethylene)-</u>
<u>1,3-dihydroindol-2-one</u>

4-Methoxy-3-phenylbenzaldehyde was condensed with 5-
chloro-2-oxindole to give 0.3 g of 5-chloro-3-(6-
methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one as a
yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 11.5 (s, 1H, CONH), 7.8-6.8 (m, 12H,
aromatic, =CH-), 3.9 (s, 3H, OCH$_3$).

Example 68: <u>3-(3-Cyclohexyl-4-methoxybenzylidene)-4-methyl-</u>
<u>1,3-dihydroindol-2-one</u>

3-Cyclohexyl4-methoxybenzaldehyde was condensed with 4-
methyl-2-oxindole to give 0.3 g of 3-(3-cyclohexyl-4-
methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one as a
yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.6 (s, 1H, CONH), 7.7-6.8 (m, 7H,
aromatic, =CH-), 3.9 (s, 3H, OCH$_3$), 3.0 (m, 1H, CH), 2.5 (s,
3H, CH$_3$), 1.8-1.4 (m, 10H, 5xCH$_2$).

Example 69: <u>3-(2,3-Dihydrobenzofuran-5-ylmethylene)-4-</u>
<u>methyl-1,3-dihydroindol-2-one</u>

2,3-Dihydro-5-formylbenzofuran (commercially available)
was condensed with 4-methyl-2-oxindole to give 0.25 g of 3-
(2,3-dihydrobenzofuran-5-ylmethylene)-4-methyl-1,3-
dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.5(s, 1H, CONH), 8.4-6.6 (m, 7H,
aromatic, =CH-), 4.6 (t, 2H, OCH$_2$), 3.2 (t, 2H, OCH$_2$), 2.5 (s,
3H, CH$_3$).

Example 70: <u>3-(3-Isopropyl-4-methoxybenzylidene)-1,3-</u>
<u>dihydropyrrolo[2,3-b]pyridin-2-one</u>

3-Isopropyl-4-methoxybenzaldehyde was condensed with 7-
aza-2-oxindole to give 0.25 g of 3-(3-isopropyl-4-methoxy-
benzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one as a
yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 11.5(s, 1H, CONH), 8.0-6.8 (m, 7H, aromatic, =CH-), 3.7 (s, 3H, OCH$_3$), 3.2 (m, 1H, CH), 1.2 (d, 6H, 2xCH$_3$).

Example 71:   3-(6-Methoxybiphenyl-3-ylmethylene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

4-Methoxy-3-phenylbenzaldehyde was condensed with 7-aza-2-oxindole to give 0.25 g of 3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.5 (s, 1H, CONH), 8.5-6.8 (m, 12H, aromatic, =CH-), 3.9 (s, 3H, OCH$_3$).

Example 72:   3-(3-Cyclohexyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

3-Cyclohexyl-4-methoxybenzaldehyde was condensed with 7-aza-2-oxindole to give 0.25 g of 3-(3-Cyclohexyl-4-methoxy-benzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one as a yellow-orange solid.

Example 73:   3-(2,3-Dihydrobenzofuran-5-ylmethylene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

2,3-Dihydro-5-formylbenzofuran (commercially available) was condensed with 7-aza-2-oxindole to give 0.2 g of 3-(2,3-dihydrobenzofuran-5-ylmethylene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.4 (s, 1H, CONH), 8.3-6.8 (m, 7H, aromatic, =CH-), 4.6 (t, 2H, OCH$_2$), 3.2 (t, 2H, OCH$_2$).

Example 74:   3-(3,5-Diisopropyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

3,5-Diisopropyl-4-methoxybenzaldehyde was methylated and then formylated. The aldehyde was condensed with 7-aza-2-oxindole to give 0.2 g of 3-(3,5-diisopropyl-4-methoxy-benzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 11.5 (s, 1H, CONH), 8.0-6.8 (m, 7H,

aromatic, =CH-), 3.7 (s, 3H, OCH₃), 3.2 (m, 2H, 2xCH), 1.2 (m, 12H, 4xCH₃).

Example 75:   5-Bromo-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

5   3-Isopropyl-4-methoxybenzaldehyde was condensed with 5-bromo-2-oxindole to give 0.3 g of 5-bromo-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

¹HNMR (d₆-DMSO) δ: 10.6 (s, 1H, CONH), 8.5, 8.4, 7.9, 7.9,
10   7.3, 7.1, 6.8 (m, 7H, aromatic, =CH-), 3.9 (m, 3H, OCH₃), 3.3 (m, 1H, CH), 1.2 (d, 6H, 2xCH₃).

Example 76:   5-Bromo-3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

          3-Cyclopentyl-4-methoxybenzaldehyde was condensed with 5-
15   bromo-2-oxindole to give 0.3 g of 5-bromo-3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

¹HNMR (d₆-DMSO) δ: 10.6 (s, 1H, CONH), 8.6-6.7 (m, 7H, aromatic, =CH-), 3.9 (s, 3H, OCH₃), 3.2 (m, 1H, CH), 1.8 (m,
20   8H, 4xCH₂).

Example 77:   5-Chloro-3-(3-cyclopentyl-4-methoxy-benzylidene)-4-methyl-1,3-dihydroindol-2-one

          A suspension of 3.0 g of 4-methyl-2-oxindole was stirred in 50 ml of acetonitrile at room temperature while 3.3 g of N-
25   chlorosuccinimide was added in portions. Trifluoroacetic acid (1 ml) was then added. The suspension was stirred at room temperature for 3 days during which time solids were always present. The solids were collected by vacuum filtration, washed with a small amount of cold acetone and dried overnight
30   in a vacuum oven at 40°C to give 2.5 g (68 %) of 5-chloro-4-methyl-2-oxindole.

          3-Cyclopentyl-4-methoxybenzaldehyde was condensed with 4-methyl-5-chloro-2-oxindole to give 0.3 g of 5-Chloro-3-(3-

cyclopentyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 78: <u>5-Chloro-3-(6-methoxybiphenyl-3-ylmethylene)-4-methyl-1,3-dihydroindol-2-one</u>

5   4-Methoxy-3-phenylbenzaldehyde was condensed with 4-methyl-5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(6-methoxybiphenyl-3-ylmethylene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 79: <u>5-Chloro-3-(5-isopropyl-4-methoxy-2-methyl-</u>
10   <u>benzylidene)-4-methyl-1,3-dihydroindol-2-one</u>

5-Isopropyl-4-methoxy-2-methylbenzaldehyde was condensed with 4-methyl-5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

15   **Example 80:** <u>5-Chloro-3-(4-methoxy-3,5-dimethylbenzylidene)-4-methyl-1,3-dihydroindol-2-one</u>

2,6-Dimethylphenol was methylated and then formylated. The aldehyde was condensed with 4-methyl-5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(4-methoxy-3,5-dimethyl-
20   benzylidene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 81: <u>3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-trifluoromethyl-1,3-dihydroindol-2-one</u>

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with
25   6-trifluoromethyl-2-oxindole to give 0.35 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-6-trifluoromethyl-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 82: <u>6-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one</u>

30   3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 6-chloro-2-oxindole to give 0.3 g of 6-chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 83: <u>3-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-</u>

<u>oxo-2,3-dihydro-1*H*-indol-5-yl]-propionic acid</u>

Potassium cyanide (2.0 g) was added to 15 ml of
dimethylsulfoxide and heated to 90°C. 5-chloroethyl-2-oxindole
(3.0 g) dissolved in 5 ml of dimethylsulfoxide was added
5   slowly with stirring, and the reaction was heated to 150°C and
stirred for 2 hours. The mixture was cooled, poured into ice
water and the precipitate which formed was collected by vacuum
filtration, washed with water, and dried to give crude
product. The crude material was chromatographed on silica gel
10   (5% methanol in chloroform) to give 1.2 g (42%) of 5-
cyanoethyl-2-oxindole.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.28 (s, br, NH), 7.12 (s,
1H, H-4), 7.07 (d, J = 8Hz, 1H), 6.74 (d, J = 8Hz, 1H), 3.43
(s, 2H, CH$_2$), 2.71-2.82 (m, 4H, 2xCH$_2$).

15   5-Cyanoethyl-2-oxindole (4.02 g) in 10 ml of water
containing 25 ml of concentrated hydrochloric acid was
refluxed for 4 hours. The mixture was cooled, water added and
the resulting solid collected by vacuum filtration, washed
with water and dried to give 1.9 g (44 % yield) of 5-
20   carboxyethyl-2-oxindole as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 12.00 (s, br, 1H, 5-
CH$_2$CH$_2$COO*H*), 10.21 (s, 1H, NH-1), 7.05 (s, 1H, H-4), 6.99 (d, J
= 9Hz, 1H, H-6), 6.69 (d, J = 9Hz, 1H, H-7), 3.40 (s, 2H, CH$_2$-
3), 2.74 (t, J = 7Hz, 2H, 5-C*H$_2$*CH$_2$COOH) and 2.46 (t, J = 7Hz,
25   2H, 5-CH$_2$C*H$_2$*COOH).

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with
5-(2-carboxyethyl)-2-oxindole to give 0.35 g of 3-[3-(3,5-
diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1*H*-indol-
5-yl]-propionic acid as a yellow-orange solid.

30   **Example 84:**   **3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-**
methoxy-1,3-dihydroindol-2-one

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with
6-methoxy-2-oxindole to give 0.3 g of 3-(3,5-diisopropyl-4-

methoxybenzylidene)-6-methoxy-1,3-dihydroindol-2-one as a
yellow-orange solid.

Example 85:   5-Butyl-3-(3,5-diisopropyl-4-methoxy-benzylidene)-1,3-dihydroindol-2-one

5   To 15 g of aluminum chloride suspended in 30 ml of 1,2-
dichloroethane in an ice bath was added 7.5 g of 2-oxindole
and then 12 g of butyryl chloride. The resulting suspension
was stirred at 50 °C overnight. The mixture was poured into
ice water and extracted 3 times with ethyl acetate.   The
10   combined ethyl acetate layers were washed with brine, dried
over sodium sulfate, and concentrated to dryness to give a
brown solid. The solid was chromatographed on silica gel (50
% ethyl acetate in hexane) to give 4.3 g (38%) of 5-butanoyl-
2-oxindole as a yellow solid.

15   ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.67 (s, br, NH), 7.84 (d, J
= 8.39Hz, 1H), s, 1H, H-4), 6.88 (d, J = 8.39Hz, 1H), 3.53 (s,
2H, CH2), 2.88 (t, J = 7.16Hz, 2H, CH2), 1.58-1.64 (m, 2H),
0.9 (t, J = 7.58Hz, 3H, CH3).

Triethylsilane (2.3 g) was added to 2 g of 5-butanoyl-2-
20   oxindole in 20 ml of trifluoroacetic acid at room temperature
and the solution stirred for 3 hours.   The reaction was
poured into ice water to give a red oil which solidified after
standing. The solid was collected by vacuum filtration,
washed with water and hexane and dried to give 1.7 g (91%
25   yield) of 5-butyl-2-oxindole as an off-white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.19 (s, br, NH), 7.01 (s,
1H, H-4), 6.95 (d, J = 8Hz, 1H), 6.69 (d, J = 8Hz, 1H), 3.4
(s, 2H, CH$_2$), 2.49 (t, J = 8Hz, 2H, CH$_2$), 1.48-1.52 (m, 2H),
1.25-1.31 (m, 2H), 0.88 (t, J = 7Hz, 3H).

30   3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with
5-butyl-2-oxindole to give 0.3 g of 5-butyl-3-(3,5-
diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a
yellow-orange solid.

Example 86:   **3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid**

Trimethylsilyldiazomethane in hexane (2 M) was added dropwise to the solution of 2-chloro-3-carboxynitrobenzene
5   (2.01 g) in methanol (20 ml) at room temperature until no further gas evolution occurred. The excess trimethylsilyl-diazomethane was quenched with acetic acid. The reaction mixture was dried by rotary pump and the residue was further dried in oven for overnight. The product (2-chloro-3-
10   methoxycarbonyl-nitrobenzene) was pure enough for the following reaction.

Dimethyl malonate (6.0 ml) was added to an ice-cold suspension of sodium hydride (2.1 g) in dimethylsulfoxide (15 ml). The reaction mixture was heated to 100°C, stirred for 1
15   hour and then cooled to room temperature. 2-Chloro-3-methoxycarbonylnitrobenzene (2.15 g) was added in one portion and the mixture was stirred at 100°C for 1.5 hour. The reaction mixture was then cooled to room temperature and poured into ice water, acidified to pH 5, and extracted with
20   ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated to give 3.0 g of the dimethyl 2-methoxycarbonyl-6-nitrophenylmalonate.

Dimethyl 2-methoxycarbonyl-6-nitrophenylmalonate (3.0 g) was refluxed in 50 ml of 6 N hydrochloric acid overnight. The
25   mixture was concentrated to dryness and refluxed for 2 hours with 1.1 g of tin(II) chloride in 20 ml of ethanol. The mixture was filtered through Celite, concentrated and chromatographed on silica gel (ethyl acetate:hexane:acetic acid) to give 0.65 g (37 % yield) of 4-carboxy-2-oxindole as a
30   white solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 12.96 (s, br, 1H, COOH), 10.74 (s, br, 1H, NH), 7.53 (d, J = 8Hz, 1H), 7.39 (t, J = 8Hz, 1H, H-6), 7.12 (d, J = 8Hz, 1H, H-7), 3.67 (s, 2H, H-3).

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 4-carboxy-2-oxindole to give 0.3 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-4-carboxylic acid as a yellow-orange solid.

5   **Example 87:**   <u>3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-(3-methoxyphenyl)-1,3-dihydroindol-2-one</u>

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 6-(3-methoxyphenyl)-2-oxindole to give 0.4 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-6-(3-methoxyphenyl)-1,3-

10   dihydroindol-2-one as a yellow-orange solid.

Example 88:   <u>7-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-dihydroindol-2-one</u>

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5-methyl-7-chloro-2-oxindole to give 0.35 g of 7-chloro-3-

15   (3,5-diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 89:   <u>[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-carbamic acid tert-butyl ester</u>

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with

20   5-tert-butyloxy-carbonylamino-2-oxindole to give 0.4 g of [3-(3,5-diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-carbamic acid tert-butyl ester as a yellow-orange solid.

Example 90:   <u>5-Chloro-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one</u>

25

3-Isopropyl-4-methoxybenzaldehyde was condensed with 5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

30   ^{1}HNMR (d$_6$-DMSO) δ: 10.6 (s, 1H, CONH), 8.5, 8.4, 7.9, 7.8, 7.2, 7.1, 6.8 (m, 7H, aromatic, =CH-), 3.9 (s, 3H, OCH$_3$), 3.3 (m, 1H, CH), 1.2(d, 6H, 2xCH$_3$).

Example 91:   <u>5-Chloro-3-(3-cyclopentyl-4-methoxy-</u>

<u>benzylidene)-1,3-dihydroindol-2-one</u>

3-Cyclopentyl-4-methoxybenzaldehyde was condensed with 5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

5

^{1}HNMR (d$_6$-DMSO) δ: 10.6 (s, 1H, CONH), 8.5, 8.3, 7.9, 7.8, 7.2, 7.1, 6.8 (m, 7H, aromatic, =CH-), 3.9 (s, 3H, OCH$_3$), 3.3 (m, 1H, CH), 1.8 (m, 8H, 4xCH$_2$).

Example 92:   **3-(6-Methoxybiphenyl-3-ylmethylene)-4-methyl-**
10 **1,3-dihydroindol-2-one**

4-Methoxy-3-phenylbenzaldehyde was condensed with 4-methyl-2-oxindole to give 0.3 g of 3-(6-methoxybiphenyl-3-ylmethylene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

15

^{1}HNMR (d$_6$-DMSO) δ: 10.5 (s, 1H, CONH), 8.3-6.7 (m, 12H, aromatic, =CH-), 3.9 (s, 3H, OCH$_3$), 2.6 (s, 3H, CH$_3$).

Example 93:   **3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-**
4-methyl-1,3-dihydroindol-2-one

5-Isopropyl-4-methoxy-2-methylbenzaldehyde was condensed
20 with 4-methyl-2-oxindole to give 0.3 g of 3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.3 (s, 1H, CONH), 8.2, 7.8, 7.1, 6.8, 6.8, 6.7 (m, 6H, aromatic, =CH-), 3.8 (s, 3H, OCH$_3$), 3.2 (m,
25 1H, CH), 2.5 (s, 3H, CH$_3$), 2.4 (s, 3H, CH$_3$), 1.2 (d, 6H, 2xCH$_3$).

Example 94:   **5-Bromo-3-(2,3-dihydrobenzofuran-5-**
ylmethylene)-1,3-dihydroindol-2-one

2,3-Dihydro-5-formylbenzofuran (commercially available)
30 was condensed with 5-bromo-2-oxindole to give 0.3 g of 5-bromo-3-(2,3-dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one as a yellow-orange solid.

^{1}HNMR (d$_6$-DMSO) δ: 10.64 (s, 1H, CONH), 7.71 (d, 1H),

7.62(vinyl, 1H), 7.61 (m, 1H), 7.52 (m, 1H), 7.37 (dd, 1H), 6.93 (d, 1H), 6.984 (d, 1H), 4.64 (t, 2H, CH$_2$), 3.22 (t, 2H, CH$_2$).

Example 95: **5-Chloro-3-(3-isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one**

3-Isopropyl-4-methoxybenzaldehyde was condensed with 4-methyl-5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(3-isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 96: **5-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one**

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 4-methyl-5-chloro-2-oxindole to give 0.4 g of 5-chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 97: **5-Chloro-3-(2,2-dimethylchroman-6-ylmethylene)-4-methyl-1,3-dihydroindol-2-one**

2,2-Dimethyl-6-formylchromane (commercially available) was condensed with 4-methyl-5-chloro-2-oxindole to give 0.3 g of 5-chloro-3-(2,2-dimethyl-chroman-6-ylmethylene)-4-methyl-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 98: **3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid**

2-Oxindole (6.7 g) was added to a suspension of aluminum chloride (23 g) in dichloroethane (30 ml) cooled in an ice bath. Chloroacetyl chloride (11.3 g) was slowly added and hydrogen chloride gas was evolved. After ten minutes of stirring, the reaction was warmed to 40 - 50°C for 1.5 hours. The mixture was cooled to room temperature and poured into ice water. The precipitate was collected by vacuum filtration, washed with water and dried under vacuum to give 10.3 g (98%) of 5-chloroacetyl-2-oxindole as an off-white solid.

A suspension of 9.3 g of 5-chloroacetyl-2-oxindole was

stirred in 90 ml pyridine at 80 - 90°C for 3 hours then cooled to room temperature. The precipitate was collected by vacuum filtration and washed with 20 ml of ethanol. The solid was dissolved in 90 ml of 2.5 N sodium hydroxide and stirred at 70

5   to 80°C for 3 hours. The mixture was cooled to room temperature and acidified to pH 2 with 0.5 N hydrochloric acid. The precipitate was collected by vacuum filtration and washed thoroughly with water to give crude 5-carboxy-2-oxindole as a dark brown solid. After standing overnight the

10   filtrate yielded 2 g of 5-carboxy-2-oxindole as a yellow solid. The crude dark brown product was dissolved in hot methanol, the insoluble material removed by filtration and the filtrate concentrated to give 5.6 g of 5-carboxy-2-oxindole as a brown solid. The combined yield was 97%.

15   ^{1}HNMR (360 MHz, DMSO-d6) δ: 2.56 (s, br, 1H, COOH-5), 10.70 (s, 1H, NH-1), 7.82 (dd, J = 2, 8Hz, 1H, H-6), 7.74 (s, br, 1H, H-4), 6.87 (d, J = 8Hz, 1H, H-7), and 3.53 (s, 2H, CH$_2$-3).

            MS m/z 178 [M+1]$^{+}$.

20   3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5-carboxy-2-oxindole to give 0.4 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid as a yellow-orange solid.

Example 99:   **3-(3,5-Diisopropyl-4-methoxybenzylidene)-5,6-**
25   **dimethoxy-1,3-dihydroindol-2-one**

            3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5,6-dimethoxy-2-oxindole to give 0.4 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-5,6-dimethoxy-1,3-dihydroindol-2-one as a yellow-orange solid.

30   **Example 100:**   **N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-**
     oxo-2,3-dihydro-1H-indol-6-yl]-methanesulfonamide

            3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 6-methylsulfonylamino-2-oxindole to give 0.4 g of N-[3-(3,5-

diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-methanesulfonamide as a yellow-orange solid.

Example 101:   *N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-benzamide*

5   3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 6-benzamido-2-oxindole to give 0.4 g of N-[3-(3,5-diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-benzamide as a yellow-orange solid.

Example 102:   *3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-(3-*
10 *ethoxyphenyl)-1,3-dihydroindol-2-one*

          Tetrakis(triphenylphosphine)palladium (0.8 g) was added to a mixture of 4.2 g of 3-ethoxyphenylboronic acid, 5.0 g of 5-bromo-2-fluoronitrobenzene and 22 ml of 2 M sodium carbonate solution in 50 ml of toluene and 50 ml of ethanol. The
15 mixture was heated to reflux for 2 hours. The cooled mixture was concentrated, water was added and the mixture was extracted twice with ethyl acetate. The ethyl acetate layer was washed with water, brine, dried, and concentrated. The residue was chromatographed on silica gel (5 % ethyl acetate
20 in hexane) to give 5.3 g (90 % yield) of crude 4-fluoro-3'-ethoxy-3-nitrobiphenyl as a yellow oil.

          Dimethyl malonate (11.4 ml) was added dropwise to 4.0 g of sodium hydride suspended in 20 ml of dimethylsulfoxide. The mixture was heated to 100°C for 10 minutes and cooled to
25 room temperature. Crude 4-fluoro-3'-ethoxy-3-nitrobiphenyl (5.3 g) in 25 ml of dimethylsulfoxide was added and the mixture was stirrede at 100°C for 2 hours. The reaction mixture was cooled and quenched with 300 ml of saturated ammonium chloride solution and extracted three times with
30 ethyl acetate. The extracts were combined, washed with water and brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 3'-ethoxy-3-nitrobiphenyl-4-malonate as a yellow oil.

Crude dimethyl 3'-ethoxy-3-nitrobiphenyl-4-malonate in 60
ml of 6 N hydrochloric acid was stirred at 100°C for 4 days and
and then cooled. The precipitate which formed was collected
by filtration, washed with water and hexane, and dried to give
5   4.7 g (77% based on 5-bromo-2-fluoronitrobenzene) of crude 3'-
ethoxy-3-nitrobiphenyl-4-acetic acid as a light tan solid.

Iron chips (2.4 g) were added in one portion to 4.6 g of
3'-ethoxy-3-nitrobiphenyl-4-acetic acid in glacial acetic acid
(40 ml). The mixture was refluxed for 2 hours. The reaction
10   mixture was cooled and concentrated to dryness. The residue
was treated repeatedly with ethyl acetate and filtered to
remove insolubles. The filtrate was washed twice with 1 N
hydrochloric acid and brine, dried over anhydrous sodium
sulfate and concentrated to give 3.5 g (91%) of 6-(3-
15   ethoxyphenyl)-2-oxindole as a light brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.4 (s, br, 1H, NH), 7.33 (t,
J = 8Hz, 1H, H-3'), 7.35 (d, J = 8Hz, 1H), 7.19 (dd, J = 1,
8Hz, 1H), 7.13 (d, J = 8Hz, 1H), 7.07-7.08 (m, 1H), 7.0 (s,
br, 1H), 6.9 (dd, J = 3, 8Hz, 1H), 4.08 (q, J = 7Hz, 2H, O
20   CH_2CH_3), 3.49 (s, 2H, CH_2), 1.34 (t, J = 7Hz, 3H, OCH_2CH_3).
MS m/z 254.2 $[M+1]^+$.

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with
6-(3-ethoxyphenyl)-2-oxindole to give 0.4 g of 3-(3,5-
diisopropyl-4-methoxybenzylidene)-6-(3-ethoxyphenyl)-1,3-
25   dihydroindol-2-one as a yellow-orange solid.

**Example 103:   3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-
phenyl-1,3-dihydroindol-2-one**

Tetrakis(triphenylphosphine)palladium (0.8 g) was added
30   to a mixture of 3.1 g of benzeneboronic acid, 5 g of 5-bromo-
2-fluoronitrobenzene and 22 ml of 2M aqueous sodium carbonate
in 50 ml of toluene and 50 ml of ethanol. The mixture was
refluxed for 2 hours, concentrated, and the residue extracted

twice with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried, and concentrated to give a yellow oil. The oil was chromatographed on silica gel (5% ethyl acetate in hexane) to give 4.75 g (96%) of 4-fluoro-3-

5   nitrobiphenyl as a yellow oil.

Dimethyl malonate (10 ml) in 25 ml of dimethylsulfoxide was added dropwise to 3.5 g of sodium hydride suspended in 25 ml of dimethylsulfoxide and the mixture stirred at 100°C for 10 minutes. The mixture was cooled to room temperature and 4.7 g

10 of 4-fluoro-3-nitrobiphenyl in 25 ml of dimethylsulfoxide was added. The mixture was stirred at 100°C for 2 hours, cooled and quenched with 300 ml of saturated ammonium chloride solution. The mixture was extracted three times with ethyl acetate and the combined organic layers washed with water and

15 brine and evaporated to give a yellow oil, crude dimethyl-3-nitrobiphenyl-4-malonate.

Crude dimethyl-3-nitrobiphenyl-4-malonate was refluxed in 30 ml of 6 N hydrochloric acid for 24 hours. The precipitate was collected by filtration, washed with water and dried to

20 give 4.5 g (80% based on 4-fluoro-3-nitrobiphenyl) of 3-nitrobiphenyl-4-acetic acid as a cream colored solid.

Iron chips (2.6 g) were added all at once to 4.5 g of 3-nitrobiphenyl-4-acetic acid in 40 ml of acetic acid. The mixture was refluxed for 2 hours, concentrated to dryness and

25 the residue taken up in ethyl acetate. The solids were removed by filtration and the filtrate washed twice with 1 N hydrochloric acid and brine and dried over anhydrous sodium sulfate. The filtrate was concentrated to give 3.4 g (93 % yield) of 6-phenyl-2-oxindole as a light brown solid.

30 ^{1}HNMR (360 MHz, DMSO-d6) δ: 10.4 (s, br, 1H, NH-1), 7.57-7.6 (m, 2H), 7.42-7.46 (m, 2H), 7.32-7.37 (m, 1H), 7.27 (d, J = 8, 1H, H-4), 7.19 (dd, J = 2 and 8Hz, 1H, H-5), 7.01 (d, J = 2Hz, 1H, H-7), 3.49 (s, 2H, CH$_2$).

MS m/z 210 [M+1]$^+$.

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 6-phenyl-2-oxindole to give 0.4 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-6-phenyl-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 104: 3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one

3,5-Diisopropyl-4-methoxybenzaldehyde was condensed with 5-fluoro-2-oxindole to give 0.3 g of 3-(3,5-diisopropyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 105: 5-Fluoro-3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-dihydroindol-2-one

3,5-Dimethyl-4-methoxybenzaldehyde was condensed with 5-fluoro-2-oxindole to give 0.3 g of 5-fluoro-3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 106: 3-(2,2-Dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one

2,2-Dimethyl-6-formylchromane (commercially available) was condensed with 2-oxindole to give 0.3 g of 3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one as a yellow-orange solid.

Example 107: 3-{2-[6-(4-Fluorophenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-5-methyl-1H-pyrrol-3-yl}propionic acid

6-(4-Fluorophenyl)-1,3-dihydroindol-2-one (70 mg, 0.31 mmol) was condensed with 3-(2-formyl-5-methyl-1H-pyrrol-3-yl)-propionic acid (56 mg) to give the title compound.

^{1}HNMR (360 MHz, DMSO-d_6) δ 13.38 (s, br, 1H, COOH), 12.08 (s, br, 1H, NH), 10.87 (s, br, 1H, NH), 7.79 (d, J = 7.9 Hz, 1H), 7.63-7.69 (m, 3H), 7.23-7.29 (m, 3H), 7.07 (d, J = 1.4 Hz, 1H), 6.06 (d, J = 2.2 Hz, 1H), 2.96 (t, J= 7.2 Hz, 2H, CH_2), 2.52 (t, J = 7.2 Hz, 2H, CH_2), 2.32 (s, 3H, CH_3).

MS-EI *m/z* 390 [M⁺].

Example 108: <u>4-(2-Carboxyethyl)-5-[6-(4-fluorophenyl)-2-oxo-1,2-dihydro-indol-3-ylidenemethyl]-2-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester</u>

6-(4-Fluorophenyl)-1,3-dihydroindol-2-one (60 mg, 0.26 mmol) was condensed with 4-(2-carboxyethyl)-5-formyl-2-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (65 mg), prepared by formylation of 4-(2-carboxyethyl)-3-ethoxycarbonyl-2-methylpyrrole (Bulter, A.R., and George, S.D. (1993) Tetrahedron <u>49</u>: 7017-7026), to give the title compound.

¹HNMR (360 MHz, DMSO-d_6) δ 13.85 (s, br, 1H, N*H*), 12.03 (s, br, 1H, COO*H*), 11.05 (s, br, 1H, N*H*), 7.83 (d, *J* = 7.9 Hz, 1H), 7.76 (s, 1H, H-vinyl), 7.66-7.70 (m, 2H), 7.25-7.30 (m, 3H), 7.08 (d, *J* = 1.4 Hz, 1H), 4.22 (q, *J* = 7.0 Hz, 2H, OC*H₂*CH₃), 3.20 (t, *J* = 7.6 Hz, 2H, C*H₂*), 2.54 (s, 3H, C*H₃*), 2.45 (m, 2H, C*H₂*), 4.22 (t, *J* = 7.0 Hz, 2H, OCH₂C*H₃*),

MS-EI *m/z* 462 [M⁺].

Example 109: <u>3-{2-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-5-methyl-1*H*-pyrrol-3-yl}-propionic acid</u>

6-(2-Methoxyphenyl)-1,3-dihydroindol-2-one (71 mg, 0.3 mmol) was condensed with 3-(2-formyl-5-methyl-1*H*-pyrrol-3-yl)-propionic acid (54 mg) to give the title compound.

¹HNMR (300 MHz, DMSO-d_6) δ 13.33 (s, br, 1H, N*H*), 12.07 (s, br, 1H, COO*H*), 10.74 (s, br, 1H, N*H*), 7.68 (d, *J* = 7.8 Hz, 1H), 7.56 (s, 1H, H-vinyl), 7.25-7.28 (m, 2H), 6.94-7.05 (m, 4H), 5.99 (br s, 1H), 3.70 (s, 3H, OC*H₃*), 2.89 (m, 2H, C*H₂*), 2.45 (m, 2H, C*H₂*), 2.26 (s, 3H, C*H₃*).

MS-EI *m/z* 402 [M⁺].

Example 110: <u>4-(2-Carboxyethyl)-5-[6-(2-methoxyphenyl)-2-oxo-1,2-dihydro-indol-3-ylidenemethyl]-2-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester</u>

¹HNMR (300 MHz, DMSO-d_6) δ 13.88 (s, br, 1H, N*H*), 12.07

(s, br, 1H, COO*H*), 10.99 (s, br, 1H, N*H*), 7.77 (d, *J* = 7.8 Hz, 1H), 7.74 (s, 1H, H-vinyl), 7.28-7.36 (m, 2H), 7.07-7.1 (m, 2H), 6.99-7.04 (m, 2H), 4.21 (q, *J* = 7.0 Hz, 2H, OC*H₂*CH₃), 3.76 (s, 3H, OC*H₃*), 3.19 (m, 2H, C*H₂*), 2.54 (s, 3H, C*H₃*), 2.50 (m, 2H, C*H₂*), 1.04 (t, *J* = 7.0 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 474 [M⁺].

Example 111: 3-[2-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-5-methyl-1*H*-pyrrol-3-yl]propionic acid

5-Chloro-1,3-dihydroindol-2-one (61 mg, 0.36 mmol) was condensed with 3-(2-formyl-5-methyl-1*H*-pyrrol-3-yl)-propionic acid (66 mg) to give the title compound.

MS-EI *m/z* 300 and 302 [M-1 and M+1].

Example 112: 4-(2-Carboxyethyl)-5-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester

¹HNMR (300 MHz, DMSO-*d₆*) δ 13.88 (s, br, 1H, N*H*), 12.04 (s, br, 1H, COO*H*), 11.08 (s, br, 1H, N*H*), 7.93 (d, *J* = 2.0 Hz, 1H), 7.83 (s, 1H, H-vinyl), 7.15 (dd, *J* = 2.0, 8.3 Hz, 1H), 6.87 (d, *J* = 8.3 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H, OC*H₂*CH₃), 3.21 (t, *J* = 7.6 Hz, 2H, C*H₂*), 2.53 (s, 3H, C*H₃*), 2.45 (t, *J* = 7.6 Hz, 2H, C*H₂*), 1.28 (t, *J* = 7.1 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 402 and 404 [M-1 and M+1].

Example 117: 4-(2-Carboxyethyl)-3-methyl-5-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester

Oxindole (0.25g, 1.88 mmol) was condensed with 4-(2-carboxyethyl)-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (0.5 g, 1.97 mmol) to give 0.45 g (65%) of the title compound.

¹HNMR (300 MHz, DMSO-*d₆*) δ 13.85 (s, br, 1H, N*H*), 12.12 (s, br, 1H, COO*H*), 10.99 (s, br, 1H, N*H*), 7.78 (d, *J* = 7.6 Hz, 1H), 7.70 (s, 1H, H-vinyl), 7.17 (m 1H), 6.99 (m, 1H), 4.25 (q, *J* = 7.1 Hz, 2H, OC*H₂*CH₃), 2.93 (t, *J* = 7.4 Hz, 2H, C*H₂*),

2.38 (t, *J* = 7.4 Hz, 2H, C*H₂*), 2.24 (s, 3H, C*H₃*), 1.29 (t, *J* = 7.1 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 368 [M⁺].

Example 118: 2-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-(3-morpholin-4-ylpropyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester

2-Oxo-2,3-dihydro-1*H*-indole-5-sulfonic acid methylamide was stirred with 5-formyl-2-methyl-4-(3-morpholin-4-ylpropyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester overnight at room temperature to give the title compound.

¹HNMR (300 MHz, DMSO-*d₆*) δ 13.86 (s, br, 1H, N*H*), 11.39 (s, br, 1H, N*H*), 8.16 (d, *J* = 1.6 Hz, 1H, H-4), 7.82 (s, 1H, H-vinyl), 7.57 (dd, *J* = 1.6 & 6.9 Hz, 1H, H-6), 7.21 (m, 1H, C*H₃*NHSO₂), 7.07 (d, *J* = 6.9 Hz, 1H, H-7), 4.21 (q, *J* = 7.0 Hz, 2H, OC*H₂*CH₃), 3.52 (m, 4H, 2xCH₂), 3.03 (m, 2H, CH₂), 2.55 (s, 3H, C*H₃*), 2.40 (d, *J* = 5.0 Hz, 3H, C*H₃*NHSO₂), 2.25 (m, 6H, 3xC*H₂*), 1.69 (m, 2H, C*H₂*), 1.29 (t, *J* = 7.0 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 516 [M⁺].

Example 119: 2-Methyl-4-[3-(4-methylpiperazin-1-yl)-propyl]-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester

¹HNMR (300 MHz, DMSO-*d₆*) δ 13.88 (s, br, 1H, N*H*), 11.38 (s, br, 1H, N*H*), 8.16 (d, *J* = 1.6 Hz, 1H, H-4), 7.79 (s, 1H, H-vinyl), 7.57 (dd, *J* = 1.6 & 6.6 Hz, 1H, H-6), 7.21 (m, 1H, C*H₃*NHSO₂), 7.05 (d, *J* = 6.6 Hz, 1H, H-7), 4.21 (q, *J* = 7.0 Hz, 2H, OC*H₂*CH₃), 3.01 (m, 2H, C*H₂*), 2.55 (s, 3H, C*H₃*), 2.40 (d, *J* = 5.4 Hz, 3H, C*H₃*NHSO₂), 2.57 (m, 10H, 5xC*H₂*), 2.09 (s, 3H, C*H₃*N), 1.67 (m, 2H, C*H₂*), 1.29 (t, *J* = 7.0 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 529 [M⁺].

Example 120: 4-(3-Dimethylaminopropyl)-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-1*H*-pyrrole-3-carboxylic acid ethyl ester

¹HNMR (360 MHz, DMSO-*d₆*) δ 13.95 (s, br, 1H, N*H*), 10.76

(s, br, 1H, N*H*), 7.70 (s, 1H, H-vinyl), 7.31 (d, *J* = 2.2 Hz, 1H, H-4), 6.78 (d, *J* = 8.3 Hz, 1H, H-7), 6.73 (dd, *J* = 2.2 & 8.3 Hz, 1H, H-6), 4.21 (q, *J* = 7.0 Hz, 2H, OC*H*$_2$CH$_3$), 3.76 (s, 3H, OC*H*$_3$), 2.98 (m, 2H, CH$_2$), 2.53 (s, 3H, CH$_3$), 2.19 (m, 2H, CH$_2$), 2.11 (s, 6H, N(CH$_3$)$_2$), 1.64 (m, 2H, CH$_2$), 1.30 (t, *J* = 7.0 Hz, 3H, OCH$_2$C*H*$_3$).

MS-EI *m/z* 411 [M$^+$].

Example 121: 5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-(3-morpholin-4-ylpropyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (300 MHz, DMSO-d_6) δ 14.0 (s, br, 1H, N*H*), 10.77 (s, br, 1H, N*H*), 7.65 (s, 1H, H-vinyl), 7.40 (m, 1H), 6.76 (m, 2H), 4.2 (q, *J* = 7.0 Hz, 2H, OC*H*$_2$CH$_3$), 3.76 (s, 3H, OC*H*$_3$), 3.54 (m, 4H, 2xCH$_2$), 2.99 (m, 2H, CH$_2$), 2.52 (s, 3H, CH$_3$), 2.25 (m, 6H, 3xCH$_2$), 1.67 (m, 2H, CH$_2$), 1.29 (t, *J* = 7.0 Hz, 3H, OCH$_2$C*H*$_3$).

MS-EI *m/z* 453 [M$^+$].

Example 122: 5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-[3-(4-methylpiperazin-1-yl)-propyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.99 (s, br, 1H, N*H*), 10.78 (s, br, 1H, N*H*), 7.63 (s, 1H, H-vinyl), 7.39 (d, *J* = 1.8 Hz, 1H, H-4), 6.76 (m, 2H), 4.2 (q, *J* = 7.1 Hz, 2H, OC*H*$_2$CH$_3$), 3.77 (s, 3H, OC*H*$_3$), 2.97 (m, 2H, CH$_2$), 2.52 (s, 3H, CH$_3$), 2.25 (m, 10H, 5xCH$_2$), 2.08 (s, 3H, NCH$_3$), 1.66 (m, 2H, CH$_2$), 1.29 (t, *J* = 7.1 Hz, 3H, OCH$_2$C*H*$_3$).

MS-EI *m/z* 466 [M$^+$].

Example 123: 2-Methyl-4-(3-morpholin-4-ylpropyl)-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.85 (s, br, 1H, N*H*), 11.33 (s, br, 1H, N*H*), 8.19 (d, *J* = 1.8 Hz, 1H, H-4), 7.75 (s, 1H, H-vinyl), 7.63 (dd, *J* = 1.8 & 8.3 Hz, 1H, H-6), 7.16 (s, 2H,

H_2NSO_2), 7.02 (d, J = 8.3 Hz, 1H, H-7), 4.21 (q, J = 7.3 Hz, 2H, OCH_2CH$_3$), 3.53 (m, 4H, 2xCH$_2$), 3.01 (m, 2H, CH$_2$), 2.55 (s, 3H, CH$_3$), 2.28 (m, 6H, 3xCH$_2$), 1.68 (m, 2H, CH$_2$), 1.29 (t, J = 7.3 Hz, 3H, OCH$_2$CH_3).

5   MS-EI m/z 502 [M$^+$].

Example 124:   2-Methyl-4-[3-(4-methylpiperazin-1-yl)-propyl]-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.85 (s, br, 1H, NH), 11.31

10   (s, br, 1H, NH), 8.18 (d, J = 1.5 Hz, 1H, H-4), 7.74 (s, 1H, H-vinyl), 7.63 (dd, J = 1.5 & 8.1 Hz, 1H, H-6), 7.15 (s, 2H, H_2NSO_2), 7.01 (d, J = 8.1 Hz, 1H, H-7), 4.21 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 3.0 (m, 2H, CH$_2$), 2.54 (s, 3H, CH$_3$), 2.26 (m, 10H, 5xCH$_2$), 2.09 (s, 3H, NCH$_3$), 1.67 (m, 2H, CH$_2$), 1.29 (t, J = 7.1

15   Hz, 3H, OCH$_2$CH_3).

      MS-EI m/z 515 [M$^+$].

Example 125:   3-[4-Ethoxycarbonyl-5-methyl-3-(3-morpholin-4-ylpropyl)-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid

20   ^{1}HNMR (300 MHz, DMSO-d_6) δ 13.88 (s, br, 1H, NH), 11.03 (s, br, 1H, COOH), 8.17 (s, 1H, H-4), 7.76 (d, J = 8.4 Hz, 1H, H-6), 7.59 (s, 1H, H-vinyl), 6.80 (d, J = 8.4 Hz, 1H, H-7), 4.21 (q, J = 7.2 Hz, 2H, OCH_2CH$_3$), 3.54 (m, 4H, 2xCH$_2$), 2.98 (m, 2H, CH$_2$), 2.49 (s, 3H, CH$_3$), 2.29 (m, 6H, 3xCH$_2$), 1.64 (m,

25   2H, CH$_2$), 1.29 (t, J = 7.2 Hz, 3H, OCH$_2$CH_3).

      MS-EI m/z 467 [M$^+$].

Example 126:   2-Methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-(3-pyrrolidin-1-ylpropyl)-1H-pyrrole-3-carboxylic acid ethyl ester

30   ^{1}HNMR (360 MHz, DMSO-d_6) δ 13.84 (s, 1H, NH), 11.28 (br s, 1H, NH), 8.06 (d, J = 2.1 Hz, 1H), 7.83 (s, 1H, H-vinyl), 7.57 (dd, J = 2.1 & 8.3 Hz, 1H), 7.20 (m, 1H, HNCH$_3$), 7.06 (d, J = 8.3 Hz, 1H), 4.22 (q, J = 7.0 Hz, 2H, OCH_2CH$_3$), 3.04 (m, 2H,

CH₂), 2.55 (s, 3H, CH₃), 2.40 (d, J = 4.8 Hz, 3H, HNCH₃), 2.3-2.36 (m, 6H, 3xCH₂), 1.66-1.71 (m, 6H, 3xCH₂), 1.30 (t, J = 7.0 Hz, 3H, OCH₂CH₃).

MS-EI *m/z* 500 [M⁺].

5 **Example 127:** 5-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2-methyl-4-(3-pyrrolidin-1-ylpropyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester

¹HNMR (300 MHz, DMSO-*d₆*) δ 13.96 (s, 1H, N*H*), 10.75 (br s, 1H, N*H*), 7.65 (s, 1H, H-vinyl), 7.28 (d, J = 2.2 Hz, 1H),
10 6.71-6.79 (m, 2H), 4.20 (q, J = 7.1 Hz, 2H, OC*H₂*CH₃), 3.75 (s, 3H, OC*H₃*), 3.0 (m, 2H, C*H₂*), 2.52 (s, 3H, C*H₃*), 2.26-2.35 (m, 6H, 3xC*H₂*), 1.65-1.71 (m, 6H, 3xC*H₂*), 1.29 (t, J = 7.1 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 437 [M⁺].

15 **Example 128:** 3-[4-Ethoxycarbonyl-5-methyl-3-(3-pyrrolidin-1-ylpropyl)-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylic acid

¹HNMR (300 MHz, DMSO-*d₆*) δ 13.80 (s, 1H, N*H*), 11.26 (br s, 1H, N*H*), 8.2 (s, 1H), 7.77 (m, 2H), 6.92 (d, J = 8.1 Hz, 1H),
20 4.23 (q, J = 6.9 Hz, 2H, OC*H₂*CH₃), 3.03 (m, 2H, C*H₂*), 2.54 (s, 3H, C*H₃*), 2.40 (m, 6H, 3xC*H₂*), 1.68 (m, 6H, 3xC*H₂*), 1.29 (t, J = 6.9 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 451 [M⁺].

Example 130: 2-Methyl-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-
25 3-ylidenemethyl)-4-(3-pyrrolidin-1-ylpropyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester

¹HNMR (300 MHz, DMSO-*d₆*) δ 13.98 (s, 1H, N*H*), 11.30 (br s, 1H, N*H*), 8.10 (d, J = 1.9 Hz, 1H), 7.79 (s, 1H, H-vinyl), 7.63 (dd, J = 1.9 & 8.2 Hz, 1H), 7.16 (br s, 2H, *H₂*NSO₂), 7.02 (d, J
30 = 8.2 Hz, 1H), 4.21 (q, J = 6.9 Hz, 2H, OC*H₂*CH₃), 3.03 (m, 2H, C*H₂*), 2.54 (s, 3H, C*H₃*), 2.33 (m, 6H, 3xC*H₂*), 1.68 (m, 6H, 3xC*H₂*), 1.29 (t, J = 6.9 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 486 [M⁺].

Example 131: 5-[4-(2-Hydroxyethyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-(3-morpholin-4-ylpropyl)-1H-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d_6) δ 13.93 (s, 1H, NH), 10.95 (br s, 1H, NH), 7.65 (s, 1H, H-vinyl), 7.06 (t, J = 7.6 Hz, 1H), 6.83 (d, J = 7.6 Hz, 1H), 6.77 (d, J = 7.6 Hz, 1H), 4.85 (br s, 1H, OH), 4.21 (q, J = 7.0 Hz, 2H, OCH_2CH$_3$), 3.73 (m, 2H, CH_2), 3.53 (m, 4H, 2xCH_2), 3.09 (m, 2H, CH_2), 2.92 (m, 2H, CH_2), 2.53 (s, 3H, CH_3), 2.31 (m, 6H, 3xCH_2), 1.68 (m, 2H, CH_2), 1.29 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3).

MS-EI m/z 467 [M$^+$].

Example 132: 5-[4-(2-Hydroxyethyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-(3-pyrrolidin-1-ylpropyl)-1H-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (360 MHz, DMSO-d_6) δ 13.93 (s, 1H, NH), 10.94 (br s, 1H, NH), 7.68 (s, 1H, H-vinyl), 7.06 (t, J = 7.3 Hz, 1H), 6.83 (d, J = 7.3 Hz, 1H), 6.77 (d, J = 7.3 Hz, 1H), 4.87 (br s, 1H, OH), 4.21 (q, J = 7.2 Hz, 2H, OCH_2CH$_3$), 3.72 (m, 2H, CH_2), 3.09 (m, 2H, CH_2), 2.94 (m, 2H, CH_2), 2.53 (s, 3H, CH_3), 2.38 (m, 6H, 3xCH_2), 1.67 (m, 6H, 3xCH_2), 1.29 (t, J = 7.2 Hz, 3H, OCH$_2$CH_3).

MS-EI m/z 451 [M$^+$].

Example 133: 4-(3-Dimethylaminopropyl)-2-methyl-5-(2-oxo-5-sulfamoyl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.73 (s, 1H, NH), 11.25 (br s, 1H, NH), 8.09 (d, J = 1.6 Hz, 1H), 7.90 (s, 1H, H-vinyl), 7.62 (dd, J = 1.6 & 8.1 Hz, 1H), 7.20 (br s, 2H, H_2NSO$_2$), 7.02 (d, J = 8.1 Hz, 1H), 4.21 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 3.01 (m, 2H, CH_2), 2.54 (s, 3H, CH_3), 2.16 (m, 2H, CH_2), 2.11 (s, 6H, N(CH_3)$_2$), 1.66 (m, 2H, CH_2), 1.29 (t, J = 7.1 Hz, 3H, OCH$_2$CH_3).

MS-EI m/z 460 [M$^+$].

Example 134: <u>3-[3-(3-Dimethylaminopropyl)-4-ethoxycarbonyl-5-methyl-1*H*-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid</u>

¹HNMR (300 MHz, DMSO-d_6) δ 13.77 (s, 1H, N*H*), 11.25 (br s, 1H, N*H*), 8.24 (s, 1H), 7.85 (s, 1H, H-vinyl), 7.76 (d, 1H), 6.82 (d, 1H), 4.21 (q, *J* = 6.9 Hz, 2H, OC*H*₂CH₃), 3.0 (m, 2H, C*H*₂), 2.54 (s, 3H, C*H*₃), 2.20 (m, 2H, C*H*₂), 2.14 (s, 6H, N(C*H*₃)₂), 1.64 (m, 2H, C*H*₂), 1.29 (t, *J* = 6.9 Hz, 3H, OCH₂C*H*₃).

MS *m/z* 426.2 [M+H].

Example 135: <u>4-(3-Dimethylaminopropyl)-5-[4-(2-hydroxyethyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester</u>

¹HNMR (300 MHz, DMSO-d_6) δ 13.95 (s, 1H, N*H*), 10.97 (br s, 1H, N*H*), 7.69 (s, 1H, H-vinyl), 7.05 (t, *J* = 7.7 Hz, 1H), 6.82 (d, *J* = 7.7 Hz, 1H), 6.75 (d, *J* = 7.7 Hz, 1H), 4.90 (br s, 1H, O*H*), 4.20 (q, *J* = 7.2 Hz, 2H, OC*H*₂CH₃), 3.72 (m, 2H, C*H*₂), 3.09 (m, 2H, C*H*₂), 2.90 (m, 2H, C*H*₂), 2.52 (s, 3H, C*H*₃), 2.20 (m, 2H, C*H*₂), 2.07 (s, 6H, N(C*H*₃)₂), 1.63 (m, 2H, C*H*₂), 1.28 (t, *J* = 7.2 Hz, 3H, OCH₂C*H*₃).

MS-EI *m/z* 425 [M⁺].

Example 136: <u>5-[4-(2-Hydroxyethyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-2-methyl-4-[3-(4-methylpiperazin-1-yl)-propyl]-1H-pyrrole-3-carboxylic acid ethyl ester</u>

¹HNMR (360 MHz, DMSO-d_6) δ 14.0 (s, 1H, N*H*), 10.94 (br s, 1H, N*H*), 7.70 (s, 1H, H-vinyl), 7.06 (t, *J* = 7.4 Hz, 1H), 6.83 (d, *J* = 7.4 Hz, 1H), 6.76 (d, *J* = 7.4 Hz, 1H), 4.77 (br s, 1H, O*H*), 4.22 (q, *J* = 7.21 Hz, 2H, OC*H*₂CH₃), 3.72 (m, 2H, C*H*₂), 3.36 (m, 2H, C*H*₂), 3.13 (m, 4H, 2xC*H*₂), 2.56 (m, 2H, C*H*₂), 2.53 (s, 3H, C*H*₃), 2.09 (m, 8H, 4xC*H*₂), 1.28 (t, *J* = 7.1 Hz, 3H, OCH₂C*H*₃).

Example 137: 4-(3-Dimethylaminopropyl)-2-methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

2-Oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide
5   (113 mg, 0.5 mmol) was condensed with 4-(3-dimethylamino-propyl)-5-formyl-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester (133 mg) to give 96 mg (40%) of the title compound.

^{1}HNMR (360 MHz, DMSO-d_6) δ 13.73 (s, br, 1H, NH), 11.32 (s, br, 1H, NH), 8.05 (d, J = 1.8 Hz, 1H, H-4), 7.92 (s, 1H,
10   H-vinyl), 7.57 (dd, J = 1.8 & 8.1 Hz, 1H, H-6), 7.22 (m, 1H, CH$_3$NHSO$_2$), 7.05 (d, J = 8.1 Hz, 1H, H-7), 4.21 (q, J = 7.1 Hz, 2H, OCH$_2$CH$_3$), 3.01 (m, 2H, CH$_2$), 2.54 (s, 3H, CH$_3$), 2.41 (d, J = 4.0 Hz, 3H, CH$_3$NHSO$_2$), 2.14 (s, 8H, CH$_2$ & N(CH$_3$)$_2$), 1.66 (m, 2H, CH$_2$), 1.29 (t, J = 7.1 Hz, 3H, OCH$_2$CH$_3$).

15   MS-EI m/z 474 [M$^+$].

Example 138: 4-(3-Dimethylaminopropyl)-2-methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

A mixture of 4-(3-dimethylaminopropyl)-5-formyl-2-methyl-
20   1H-pyrrole-3-carboxylic acid ethyl ester (300 mg, 1.13 mmol) and sodium hydroxide in MeOH (4 mL)/water (2 mL) was stirred at 80°C for 19 hr. The reaction was then diluted with EtOAc. The aqueous layer was separated, neutralized and concentrated. The residue was dissolved in MeOH, filtered and the filtrate
25   was concentrated to give 251 mg (94%) of 4-(3-dimethylaminopropyl)-5-formyl-2-methyl-1H-pyrrole-3-carboxylic acid.

^{1}HNMR (300 MHz, DMSO-d_6) δ 11.88 (br s, 1H, NH), 9.50 (s, 1H, CHO), 2.96 (m, 2H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.22 (m, 2H,
30   CH$_2$), 2.14 (s, 6H, N(CH$_3$)$_2$), 1.68 (m, 2H, CH$_2$CH$_2$ CH$_2$).

MS-EI m/z 238 [M$^+$].

2-Oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide
(113 mg) was condensed with 4-(3-dimethylaminopropyl)-5-

formyl-2-methyl-1H-pyrrole-3-carboxylic acid (119 mg) to give
50 mg 4-(3-dimethylaminopropyl)-2-methyl-5-(5-methylsulfamoyl-
2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-
carboxylic acid.

5   ^{1}HNMR (360 MHz, DMSO-d_6) δ 13.52 (s, br, 1H, NH), 11.25
(s, br, 1H, COOH), 8.01 (d, J = 1.7 Hz, 1H, H-4), 7.84 (s, 1H,
H-vinyl), 7.52 (dd, J = 1.7 & 8.3 Hz, 1H, H-6), 7.22 (m, 1H,
CH_3NHSO$_2$), 7.05 (d, J = 8.3 Hz, 1H, H-7), 3.08 (m, 2H, CH_2),
2.56 (s, 3H, CH_3), 2.40 (m, 3H, CH_3NHSO$_2$), 2.17 (m, 8H, CH_2 &
10   N(CH_3)$_2$), 1.72 (m, 2H, CH_2).

     MS-EI m/z 446 [M$^+$].

Example 139:   <u>**4-(2-Carboxyethyl)-3-methyl-5-(4-methyl-2-oxo-
1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
ethyl ester**</u>

15   ^{1}HNMR (300 MHz, DMSO-d_6) δ 13.97 (s, br, 1H, NH), 12.15 (
br s, 1H, COOH), 11.03 (s, 1H, NH), 7.69 (s, 1H, H-vinyl),
7.10 (t, J = 7.5 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 6.76 (d, J
= 7.5 Hz, 1H), 4.28 (q, J = 7.0 Hz, 2H, OCH_2CH$_3$), 2.88 (t, 2H,
CH_2), 2.62 (s, 3H, CH_3), 2.43 (t, 2H, CH_2), 2.26 (s, 3H, CH_3),
20   1.31 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3).

     MS-EI m/z 382 [M$^+$].

Example 140:   <u>**4-(2-Carboxyethyl)-5-(5-methoxy-2-oxo-1,2-
dihydroindol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic
acid ethyl ester**</u>

25   ^{1}HNMR (300 MHz, DMSO-d_6) δ 13.97 (s, br, 1H, NH), 12.14
(br s, 1H, COOH), 10.81 (s, 1H, NH), 7.71 (s, 1H, H-vinyl),
7.46 (m, 1H), 6.78 (m, 2H), 4.28 (q, J = 7.0 Hz, 2H, OCH_2CH$_3$),
3.76 (s, 3H, OCH_3), 2.97 (t, 2H, CH_2), 2.40 (t, 2H, CH_2), 2.26
(s, 3H, CH_3), 1.31 (t, J = 7.0 Hz, 3H, OCH$_2$CH_3).
30   MS-EI m/z 398 [M$^+$].

Example 141: <u>4-(2-Carboxyethyl)-5-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester</u>

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.70 (s, br, 1H, N*H*), 12.11 (br s, 1H, COO*H*), 10.95 (s, 1H, N*H*), 7.0 (d, *J* = 8.4 Hz, 1H, H-4), 7.55 (s, 1H, H-vinyl), 6.59 (dd, *J* = 2.7 & 8.4 Hz, 1H, H-5), 6.45 (d, *J* = 2.7 Hz, 1H, H-7), 4.27 (q, *J* = 7.0 Hz, 2H, OC*H₂*CH₃), 3.76 (s, 3H, OC*H₃*), 2.92 (t, 2H, C*H₂*), 2.39 (t, 2H, C*H₂*), 2.25 (s, 3H, C*H₃*), 1.31 (t, *J* = 7.0 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 398 [M⁺].

Example 142: <u>5-(6-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-(2-carboxy-ethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester</u>

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.77 (s, br, 1H, N*H*), 12.13 (br s, 1H, COO*H*), 11.14 (s, 1H, N*H*), 7.77 (br s, 2H), .12 (m, 2H), 4.28 (m, 2H, OC*H₂*CH₃), 2.95 (m, 2H, C*H₂*), 2.40 (m, 2H, C*H₂*), 2.26 (s, 3H, C*H₃*), 1.29 (m, 3H, OCH₂C*H₃*).

MS-EI *m/z* 446 & 448 [M-1 & M+1].

Example 143: <u>5-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-(2-carboxy-ethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester</u>

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.86 (s, br, 1H, N*H*), 12.2 (br s, 1H, COO*H*), 11.12 (s, 1H, N*H*), 8.11 (d, *J* = 2.0 Hz, 1H, H-4), 7.83 (s, 1H, H-vinyl), 7.33 (dd, *J* = 2.0 & 8.3 Hz, 1H, H-6), 6.83 (d, *J* = 8.3 Hz, 1H, H-7), 4.28 (q, *J* = 7.0 Hz, 2H, OC*H₂*CH₃), 2.98 (t, 2H, C*H₂*), 2.39 (m, 2H, C*H₂*), 2.25 (s, 3H, C*H₃*), 1.31 (t, *J* = 7.0 Hz, 3H, OCH₂C*H₃*).

MS-EI *m/z* 446 & 448 [M-1 & M+1].

Example 144: <u>4-(2-Carboxyethyl)-3-methyl-5-(2-oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester</u>

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.86 (s, br, 1H, N*H*), 12.2 (br s, 1H, COO*H*), 11.11 (s, 1H, N*H*), 7.89 (d, *J* = 7.7 Hz, 1H),

7.77 (s, 1H, H-vinyl), 7.65 (m, 2H), 7.46 (t, 2H), 7.32-7.39 (m, 2H), 7.12 (d, $J = 1.6$ Hz, 1H), 4.29 (q, $J = 7.0$ Hz, 2H, OCH_2CH$_3$), 2.97 (m, 2H, CH_2), 2.42 (m, 2H, CH_2), 2.27 (s, 3H, CH_3), 1.31 (t, $J = 7.0$ Hz, 3H, OCH$_2$CH_3).

5   MS-EI m/z 444 [M$^+$].

Example 145: 4-(2-Carboxyethyl)-3-methyl-5-(2-oxo-5-sulfamoyl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester

   ^{1}HNMR (300 MHz, DMSO-d_6) δ 13.84 (s, br, 1H, NH), 11.0 (
10   br s, 2H), 8.26 (d, $J = 1.6$ Hz, 1H, H-4), 7.88 (s, 1H, H-vinyl), 7.67 (dd, $J = 1.6$ & 8.4 Hz, 1H, H-6), 7.22 (br m, 2H, H_2NSO$_2$), 7.03 (d, $J = 8.4$ Hz, 1H, H-7), 4.29 (q, $J = 7.1$ Hz, 2H, OCH_2CH$_3$), 2.97 (t, 2H, CH_2), 2.37 (m, 2H, CH_2), 2.26 (s, 3H, CH_3), 1.31 (t, $J = 7.1$ Hz, 3H, OCH$_2$CH_3).

15   MS-EI m/z 447 [M$^+$].

Example 146: 4-(2-Carboxyethyl)-3-methyl-5-(5-methylsulfamoyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester

   ^{1}HNMR (300 MHz, DMSO-d_6) δ 13.89 (s, br, 1H, NH), 12.2 (
20   br s, 1H, COOH), 11.43 (s, 1H, NH), 8.25 (d, $J = 1.8$ Hz, 1H, H-4), 7.94 (s, 1H, H-vinyl), 7.62 (dd, $J = 1.8$ & 8.4 Hz, 1H, H-6), 7.27 (m, 1H, HNSO$_2$), 7.07 (d, $J = 8.4$ Hz, 1H, H-7), 4.29 (q, $J = 7.1$ Hz, 2H, OCH_2CH$_3$), 3.01 (t, 2H, CH_2), 2.45 (m, 2H, CH_2), 2.42 (d, $J = 5.3$ Hz, 3H, NCH_3), 2.27 (s, 3H, CH_3), 1.32
25   (t, $J = 7.1$ Hz, 3H, OCH$_2$CH_3).

   MS-EI m/z 461 [M$^+$].

Example 147: 4-(2-Carboxyethyl)-5-(5-dimethylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester

30   ^{1}HNMR (300 MHz, DMSO-d_6) δ 13.88 (s, br, 1H, NH), 12.14 (br s, 1H, COOH), 11.47 (s, 1H, NH), 8.26 (d, $J = 1.8$ Hz, 1H, H-4), 8.0 (s, 1H, H-vinyl), 7.58 (dd, $J = 1.8$ & 7.9 Hz, 1H, H-6), 7.10 (d, $J = 7.9$ Hz, 1H, H-7), 4.29 (q, $J = 7.2$ Hz, 2H,

OCH_2CH_3), 3.03 (t, 2H, CH_2), 2.61 (s, 6H, N(CH_3)$_2$), 2.42 (t, 2H, CH_2), 2.26 (s, 3H, CH_3), 1.32 (t, J = 7.2 Hz, 3H, OCH$_2$CH_3).

MS-EI m/z 489 [M$^+$].

Example 148: <u>4-(2-Carboxyethyl)-5-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester</u>

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.84 (s, br, 1H, NH), 11.0 (br s, 2H, COOH & NH), 8.23 (d, J = 1.5 Hz, 1H, H-4), 7.92 (s, 1H, H-vinyl), 7.64 (dd, J = 1.5 & 8.4 Hz, 1H, H-6), 7.43 (br m, 1H, HNC(CH$_3$)$_2$), 7.05 (d, J = 8.4 Hz, 1H, H-7), 4.29 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 2.96 (t, 2H, CH_2), 2.34 (t, 2H, CH_2), 2.25 (s, 3H, CH_3), 1.31 (t, J = 7.1 Hz, 3H, OCH$_2$CH_3), 0.95 (d, J = 6.6 Hz, 6H, HNC(CH_3)$_2$).

MS-EI m/z 489 [M$^+$].

Example 149: <u>3-[3-(3-Dimethylaminopropyl)-1H-indol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide</u>

2-Oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide (98 mg, 0.43 mmol) was condensed with 3-(3-dimethylamino-propyl)-1H-indole-2-carbaldehyde (100 mg) to give the title compound as an orange solid.

^{1}HNMR (300 MHz, DMSO-d_6) δ 12.90 (s, 1H, NH), 11.40 (br s, 1H, NH), 8.17 (d, J = 1.5 Hz, 1H), 8.15 (s, 1H, H-vinyl), 7.68 (d, J = 8.4 Hz, 1H), 7.63 (dd, J = 1.5 & 8.4 Hz, 1H), 7.54 (d, J = 8.7 Hz, 1H), 7.30 (m, 2H), 7.08 (m, 2H), 3.15 (t, J = 6.6 Hz, 2H, CH_2), 2.43 (d, J = 4.2 Hz, 3H, NCH_3), 2.17 (t, J = 6.6 Hz, 2H, CH_2), 2.14 (s, 6H, N(CH_3)$_2$), 1.78 (m, 2H, CH_2).

MS-EI m/z 438 [M$^+$].

Example 150: <u>3-[3-(3-Dimethylaminopropyl)-1H-indol-2-ylmethylene]-4-(2-hydroxyethyl)-1,3-dihydroindol-2-one</u>

^{1}HNMR (300 MHz, DMSO-d_6) δ 13.12 (s, 1H, NH), 11.01 (br s, 1H, NH), 7.95 (s, 1H, H-vinyl), 7.66 (d, J = 7.9 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.26 (t, J = 7.54 Hz, 1H), 7.11 (t, J =

7.9 Hz, 1H), 7.06 (t, *J* = 7.4 Hz, 1H), 6.87 (d, *J* = 7.4 Hz,

1H), 6.78 (d, *J* = 7.4 Hz, 1H), 4.91 (br s, 1H, O*H*), 3.79 (m,

2H, C*H*₂), 3.16 (m, 2H, C*H*₂), 3.04 (m, 2H, C*H*₂), 2.21 (m, 2H,

C*H*₂), 2.09 (s, 6H, N(C*H*₃)₂), 1.77 (m, 2H, C*H*₂).

5   MS *m/z* 389 [M⁺].

Example 151:   3-[3-(3-Dimethylaminopropyl)-1H-indol-2-ylmethylene]-5-methoxy-1,3-dihydroindol-2-one

¹HNMR (300 MHz, DMSO-*d₆*) δ 13.11 (s, 1H, N*H*), 10.84 (br s,

1H, N*H*), 7.92 (s, 1H, H-vinyl), 7.65 (d, *J* = 8.1 Hz, 1H), 7.50

10   (d, *J* = 8.1 Hz, 1H), 7.44 (s, 1H), 7.26 (t, *J* = 7.5 Hz, 1H),

7.05 (t, *J* = 7.5 Hz, 1H), 6.79 (s, 2H), 3.79 (s, 3H, OC*H*₃),

3.11 (m, 2H, C*H*₂), 2.20 (m, 2H, C*H*₂), 2.12 (s, 6H, N(C*H*₃)₂),

1.76 (m, 2H, C*H*₂).

MS *m/z* 375 [M⁺].

15   **Example 152: 5-Methyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one**

3-Methylindole-2-carbaldehyde was prepared as described

in the literature: 1) David W.M. Benzies, Pilar Matinez

Fresneda and R. Alan Jones, *Synthetic Communications*, 16 (14),

20   1799-1807 (1986) and 2) Chatterjee, A. and Biswas, K.M., *J.*

Org. Chem., 1973, 38, 4002.

A mixture of 5-methyl-2-oxindole (59 mg),

3-methylindole-2-carbaldehyde (56 mg) and piperidine (30 mg)

in ethanol(1 mL) was held in a sealed tube at 90°C overnight.

25   The mixture was cooled to room temperature. The solid was

collected by vacuum filtration, washed with cold ethanol and

dried in a vacuum oven to give 72 mg (74%) of 5-methyl-3-

(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one.

¹H NMR (360 MHz, DMSO-d6) 13.03 (s, br, 1H, NH), 10.88 (s,

30   br, 1H, NH), 7.84 (s, 1H, vinyl), 7.71 (s, br, 1H), 6.62 (d,

J = 8Hz, 1H), 7.48 (d, *J* = 8Hz, 1H), 7.26 (t, *J* = 7Hz, 1H),

7.06 (t, *J* = 7Hz, 1H), 7.0 (d, *J* = 8Hz, 1H), 6.78 (d, *J* = 8Hz,

1H), 2.59 (s, 3H, C*H*₃), 2.32 (s, 3H, C*H*₃).

MS m/z 289 [M+1]⁺.

Example 153:   3-(3-Methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid amide

A mixture of 5-aminosulfonyl-2-oxindole (84 mg),

5 3-methylindole-2-carbaldehyde (56 mg) (prepared according to *Synthetic Communications*, 1986, 16, 1799) and piperidine (30 mg) in ethanol (1 mL) was held in a sealed tube at 90°C overnight. The mixture was cooled to room temperature. The solid was collected by vacuum filtration, washed with cold

10 ethanol and dried in a vacuum oven to give 88 mg (71% yield) of 3-(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid amide.

^{1}H NMR (360 MHz, DMSO-d6) 12.86 (s, br, 1H, NH), 11.3 (s, br, 1H, NH), 8.3 (d, J = 2Hz, 1H), 7.97 (s, 1H, vinyl), 7.63

15 (dd, J = 2 and 8Hz, 1H), 7.6 (d, J = 8Hz, 1H), 7.47 (d, J = 8Hz, 1H), 7.24 (t, J = 7.5Hz, 1H), 7.12 (s, br, 2H, NH₂), 7.02 (t, J = 7.5Hz, 1H), 6.98 (d, J = 8Hz, 1H), 2.57 (s, 3H, CH₃).

MS m/z 354.1 [M+1]⁺

Example 154:   3-(3-Methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-

20 **dihydro-1H-indole-5-sulfonic acid methylamide**

A suspension of 5-chlorosulfonyl-2-oxindole (3.38 g) in 2M methylamine in tetrahydrofuran (10 mL) was stirred at room temperature for 4 hours at which time a white solid had formed. The solid was collected by vacuum filtration, washed

25 twice with 5 mL of water and dried under vacuum at 40°C to give 3.0 g (88% yield) of 5-methylaminosulfonyl-2-oxindole.

^{1}H NMR (300 MHz, DMSO-d6) δ 10.87 (s, br, 1H, NH-1), 7.86 (s, br, 1H, 5-SO₂NHCH₃),7.61 (d, J = 8Hz 1H, H-6), 7.32 (d, J = 5Hz, 1H, H-4), 6.97 (d, J = 8Hz, 1H, H-7), 2.53 (s, 2H, CH₂-3),

30 and 2.36 (s, 3H, 5-SO₂NHCH₃).

MS m/z 226.

A mixture of 5-methylaminosulfonyl-2-oxindole (90 mg), 3-methylindole-2-carbaldehyde (56 mg) (prepared according to

Synthetic Communications, 1986, 16, 1799) and piperidine
(30 mg) in ethanol (1 mL) was held in a sealed tube at 90°C
overnight. The mixture was cooled to room temperature. The
solid was collected by vacuum filtration, washed with cold
5   ethanol and dried in a vacuum oven to give 90 mg (70% yield)
of 3-(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-
indole-5-sulfonic acid methylamide (mixture of isomers).

      ¹H NMR (360 MHz, DMSO-d6) 11.13 (s, br, 1H, NH), 10.94 (s,
br, 1H, NH), 8.84 (d, J = 16Hz, 1H), 7.98 (s, 1H), 6.97-7.66
10   (m, 6H, Ar-H), 2.6 (s, 3H, CH₃), 2.4 (d, J = 5Hz, 3H, CH₃N.

Example 155:   **3-(3-Methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-
dihydro-1H-indole-5-sulfonic acid dimethylamide**

      A mixture of 5-dimethylaminosulfonyl-2-oxindole (96 mg),
3-methylindole-2-carbaldehyde (56 mg) (prepared according to
15   *Synthetic Communications,* 1986, 16, 1799) and piperidine
(30 mg) in ethanol (1 mL) was held in a sealed tube at 90°C
overnight. The mixture was cooled to room temperature. The
solid was collected by vacuum filtration, washed with cold
ethanol and dried in a vacuum oven to give 76 mg (57% yield)
20   of 3-(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-
indole-5-sulfonic acid dimethylamide (mixture of isomers).

      ¹H NMR (360 MHz, DMSO-d6) 11.09 (s, br, 1H, NH), 10.95 (s,
br, 1H, NH), 8.78 (d, J = 16Hz, 1H), 7.8 (s, 1H, vinyl),
7.45-7.54 (m, 2H), 7.32 (d, J = 8Hz, 1H), 7.1 (t, 1H), 6.98
25   (d, J = 8Hz, 1H), 6.93 (t, 1H), 2.56 (s, 6H, 2 x CH₃), 2.39 (s,
3H, CH₃)

Example 156:   **3-(3-Methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-
dihydro-1H-indole-5-carboxylic acid (piperidine salt)**

      A mixture of 5-carboxy-2-oxindole (113 mg),
30   3-methylindole-2-carbaldehyde (56 mg) (prepared according to
Synthetic Communications, 1986, 16, 1799) and piperidine (30
mg) in ethanol (1 mL) was held in a sealed tube at 90°C
overnight. The mixture was cooled to room temperature. The
solid was collected by vacuum filtration, washed with cold

ethanol and dried in a vacuum oven to give 75 mg (58% yield) of 3-(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid (piperidine salt).

   5   ^{1}H NMR (360 MHz, DMSO-d6) 13.0 (s, br, 1H, NH), 8.35 (d, J = 1.5Hz, 1H, H-4), 7.91 (s, 1H, vinyl), 7.82 (dd, J = 1.5 and 8Hz, 1H, H-6), 7.63 (d, J = 8Hz, 1H), 7.49 (d, J = 8Hz, 1H), 7.26 (t, J = 7.5Hz, 1H), 7.06 (t, J = 7.5Hz, 1H), 6.87 (d, J = 8Hz, 1H), 2.91 (t, 4H, piperidine), 2.6 (s, 3H, CH$_3$), 1.6 (m, 4H, piperidine), 1.54 (m, 2H, piperidine).

10   **Example 157: 5-Acetyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one**

   2-Oxindole (3 g) was suspended in 1,2-dichloroethane and 3.2 mL of acetyl chloride slowly added. The resulting suspension was stirred at 50°C for 5 hours, cooled, and poured

15   into water. The resulting precipitate was collected by vacuum filtration, washed copiously with water and dried under vacuum to give 2.9 g (73% yield) of 5-acetyl-2-oxindole as a brown solid.

   ^{1}H NMR (360 MHz, DMSO-d6) δ 10.75 (s, br, NH), 7.83 (d,

20   J = 8Hz, 1H), 7.78 (s, 1H, H-4), 6.88 (d, J = 8Hz, 1H), 3.53 (s, 2H, CH$_2$), 2.49 (s, 3H, CH$_3$).

   A mixture of 5-acetyl-2-oxindole (70 mg), 3-methylindole-2-carbaldehyde (56 mg) (prepared according to *Synthetic Communications*, 1986, 16, 1799) and piperidine (30

25   mg) in ethanol (1 mL) was held in a sealed tube at 90°C overnight. The mixture was cooled to room temperature. The solid was collected by vacuum filtration, washed with cold ethanol and dried in a vacuum to give 83 mg (75% yield) of 5-acetyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-

30   dihydro-indol-2-one.

   ^{1}H NMR (360 MHz, DMSO-d6) 12.86 (s, br, 1H, NH), 11.29 (s, br, 1H, NH), 8.43 (d, J = 1.5Hz, 1H, H-4), 8.01 (s, 1H, vinyl), 7.78 (dd, J = 1.5 and 8Hz, 1H, H-6), 7.58 (d, J = 8Hz,

1H), 7.45 (d, J = 8Hz, 1H), 7.23 (t, 1H), 7.01 (t, 1H), 6.92
(d, J = 8Hz, 1H), 2.58 (s, 3H, CH$_3$), 2.54 (s, 3H, CH$_3$).

MS m/z 317.2 [M+1]$^+$.

Example 158: **5-Acetyl-3-(1H-indol-2-ylmethylene)-1,3-dihydro-**

5   **indol-2-one**

Indole-2-carbaldehyde was prepared as described in the
literature: Michel Barbier, Michel Devys, Christiane Tempête,
Albert Kollmann and Jean-François Bousquet, *Synthetic
Communications*, 23(22), 3109-3117 (1993).

10   A mixture of 5-acetyl-2-oxindole (88 mg), indole-2-
carbaldehyde (87 mg) and piperidine (4 mg) in ethanol (2 mL)
was held in a sealed tube at 90°C for 3 hours. The mixture
was cooled to room temperature. The solid which formed was
collected by vacuum filtration, washed with cold ethanol and

15   dried in a vacuum oven to give 133 mg (88% yield) of
5-acetyl-3-(1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one as
an orange solid.

^{1}H NMR (360 MHz, DMSO-d6) 12.81 (s, br, 1H, NH), 11.38 (s,
br, 1H, NH), 8.38 (d, J = 1Hz, H-4), 8.17 (s, 1H, vinyl), 7.88

20   (dd, J = 1 and 8Hz, 1H, H-6), 7.69 (d, J = 8Hz, 1H), 7.58 (d,
J = 8Hz, 1H), 7.29 (t, 1H), 7.21 (s, 1H), 7.09 (t, 1H), 7.0
(d, J = 8Hz, 1H), 2.59 (s, 3H, CH$_3$).

MS m/z 303.1 [M+1]$^+$.

Example 159: **3-(1H-Indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-**

25   **indole-5-sulfonic acid amide**

A mixture of 5-aminosulfonyl-2-oxindole (106 mg),
indole-2-carbaldehyde (87 mg) (prepared according to *Synthetic
Communications*, 1993, 23, 3109) and piperidine (4 mg) in
ethanol (2 mL) was held in a sealed tube at 90°C for 3 hours.

30   The mixture was cooled to room temperature. The solid was
collected by vacuum filtration, washed with cold ethanol and
dried in a vacuum oven to give 140 mg (83% yield) of 3-(1H-
indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic
acid amide as an orange solid.

^{1}H NMR (360 MHz, DMSO-d6) 12.81 (s, br, 1H, NH), 11.38 (s, br, 1H, NH), 8.18 (d, J = 1Hz, H-4), 8.12 (s, 1H, vinyl), 7.7 (m, 2H), 7.6 (d, J = 8Hz, 1H), 7.26-7.29 (m, 2H), 7.2 (s, 2H, NH$_2$), 7.05-7.11 (m, 2H).

5   MS m/z 340 [M+1]$^+$.

Example 160: <u>5-Amino-3-(1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one</u>

A mixture of 5-*tert*-butoxycarbonylamino-2-oxindole (124 mg), indole-2-carbaldehyde (87 mg) (prepared according to
10   *Synthetic Communications*, 1993, 23, 3109) and piperidine (4 mg) in ethanol (2 mL was held in a sealed tube at 90°C for 3 hours. The mixture was cooled to room temperature. The solid was collected by vacuum filtration, washed with cold ethanol and dried in a vacuum overn to give 196 mg of
15   5-*tert*-butoxycarbonylamino-3- (1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one as a bright orange solid. The prodcuct was then dissolved in trifluoroacetic acid/dichloromethane (2 mL each) and stirred at room temperature for 1 hour. The reaction mixture was then
20   concentrated. The residue was dissolved in water and basified with saturated sodium bicarbonate solution. The solid was collected by vacuum filtration, washed with water and dried in a vacuum oven to give 129 mg of 5-amino-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one as a brown solid.

25   ^{1}H NMR (360 MHz, DMSO-d6) 13.1 (s, br, 1H, NH), 10.57 (s, br, 1H, NH), 7.65 (s, 1H, vinyl), 7.64 (d, J = 8Hz, 1H), 7.54 (d, J = 8Hz, 1H), 7.24 (t, 1H), 7.1 (s, 1H), 7.06 (t, 1H), 6.93 (d, J = 2Hz, 1H), 6.61 (d, J = 8Hz, 1H), 6.5 (dd, J = 2 and 8Hz, 1H), 4.8 (s, br, 2H, NH$_2$).

30   MS m/z 276.1 [M+1]$^+$.

Example 161: <u>3-(1H-Indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid</u>

A mixture of 5-carboxy-2-oxindole (88.5 mg), indole-2-carbaldehyde (87 mg) (prepared according to *Synthetic*

Communications, 1993, 23, 3109) and piperidine (1 drop) in ethanol (3 mL) was held in a sealed tube at 95°C for 4 hours. The mixture was cooled to room temperature. The solid was filtered and acidified with 2N hydrochloric acid. The

5　resulting solid was collected by vacuum filtration, washed with cold ethanol and dried in vacuum oven to give 60 mg (40% yield) of 3-(1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid as a mustard-colored solid.

^{1}H NMR (360 MHz, DMSO-d6) 12.8 (s, br, 1H, NH), 12.69 (s,

10　1H, COOH), 11.35 (s, br, 1H, NH), 8.33 (s, 1H), 8.16 (s, 1H), 7.86 (dd, J = 1 and 8Hz, 1H), 7.68 (d, J = 8Hz, 1H), 7.59 (d, J = 8Hz, 1H), 7.28 (t, 1H), 7.21 (s, 1H), 7.08 (t, 1H), 6.99 (d, J = 8Hz, 1H).

MS m/z 304 [M]$^+$.

15　**Example 162:** <u>6-Chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one</u>

A mixture of 6-chloro-2-oxindole (41 mg) (commercially available), indole-2-carbaldehyde (42 mg) (prepared according to *Synthetic Communications*, 1993, 23, 3109) and piperidine

20　(1 drop) in ethanol (3 mL) was held in a sealed tube at 95°C for 3 hours. The mixture was cooled to room temperature. The solid was collected by vacuum filtration, washed with cold ethanol and dried in vacuum oven to give 62 mg (87% yield) of 6-chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one as

25　a shiny orange solid.

^{1}H NMR (360 MHz, DMSO-d6) 12.83 (s, br, 1H, NH), 11.17 (s, br, 1H, NH), 7.98 (s, 1H), 7.74 (d, J = 8Hz, 1H), 7.67 (d, J = 8Hz, 1H), 7.58 (d, J = 8Hz, 1H), 7.28 (m, 1H), 7.15 (s, br, 1H), 7.08 (m, 2H), 6.91 (d, J = 2Hz, 1H).

30　MS m/z 293 and 295.

Example 163: <u>3-(1H-Indol-2-ylmethylene)-1,3-dihydro-indol-2-one</u>

A mixture of 2-oxindole (133 mg), indole-2-carbaldehyde (174 mg) (prepared according to *Synthetic Communications*,

1993, 23, 3109) and piperidine (10 mg) in ethanol was held in a sealed tube at 95°C for 4 hours. The mixture was cooled to room temperature. The solid was collected by vacuum filtration, washed with cold ethanol and dried in a vacuum

5   oven to give 3-(1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one.

^{1}H NMR (360 MHz, DMSO-d6) δ 12.97 (s, br, 1H, NH), 11.01 (s, br, 1H, NH), 7.92 (s, 1H, H-vinyl), 7.72 (d, J = 7Hz, 1H), 7.66 (d, J = 7Hz, 1H), 7.57 (dd, J = 1, 8Hz, 1H), 7.27 (dt,

10   J = 1, 7Hz, 1H), 7.21 (dt, J = 1, 8Hz, 1H), 7.13 (s, 1H), 7.01-7.1 (m, 2H), 6.91 (d, J = 8Hz, 1H).

MS El 260.

Example 164: <u>5-Chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one</u>

15   A mixture of 5-chloro-2-oxindole (167 mg), indole-2-carbaldehyde (174 mg) (prepared according to *Synthetic Communications*, 1993, 23, 3109) and piperidine (10 mg) in ethanol was held in a sealed tube at 95°C for 4 hours. The mixture was cooled to room temperature. The solid was

20   collected by vacuum filtration, washed with cold ethanol and dried in a vacuum oven to give 5-chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one.

^{1}H NMR (360 MHz, DMSO-d6) δ 12.88 (s, br, 1H, NH), 11.12 (s, br, 1H, NH), 8.07 (s, 1H, H-vinyl), 7.86 (d, J = 2Hz, 1H),

25   7.68 (d, J = 8Hz, 1H), 7.59 (dd, J = 1, 8Hz, 1H), 7.28 (m, 1H), 7.24 (dd, J = 2, 8Hz, 1H), 7.14 (s, 1H), 7.09 (dt, J = 1, 8Hz, 1H), 6.90 (d, J = 8Hz, 1H).

MS El 294 and 296.

Example 165: <u>5-Bromo-3-(1H-indol-2-ylmethylene)-1,3-dihydro-</u>

30   **<u>indol-2-one</u>**

A mixture of 5-bromo-2-oxindole (212 mg), indole-2-carbaldehyde (174 mg) (prepared according to *Synthetic Communications*, 1993, 23, 3109) and piperidine

(10 mg) in ethanol was held in a sealed tube at 95°C for 4
hours. The mixture was cooled to room temperature. The solid
was collected by vacuum filtration, washed with cold ethanol
and dried in a vacuum oven to give 5-bromo-3-(1H-indol-2-
5   ylmethylene)-1,3-dihydroindol-2-one.

        ^{1}HNMR (360 MHz, DMSO-d6) δ 12.87 (s, br, 1H, NH), 11.12
(s, br, 1H, NH), 8.07 (s, 1H, H-vinyl), 7.98 (d, J = 2Hz, 1H),
7.68 (d, J = 8Hz, 1H), 7.59 (d, J = 8Hz, 1H), 7.36 (dd, J = 2,
8Hz, 1H), 7.28 (dt, J = 1, 8Hz, 1H), 7.14 (s, 1H), 7.08 (dt,
10 J = 1, 8Hz, 1H), 6.86 (d, J = 8Hz, 1H).

        MS El 338 and 340.

Example 166: <u>3-(1H-indol-2-ylmethylene)-4-methyl-1,3-dihydro-</u>
<u>indol-2-one</u>

        A mixture of 4-methyl-2-oxindole (147 mg),
15 indole-2-carbaldehyde (174 mg) (prepared according to
Synthetic Communications, 1993, 23, 3109) and piperidine
(10 mg) in ethanol was held in a sealed tube at 95°C for 4
hours. The mixture was cooled to room temperature. The solid
was collected by vacuum filtration, washed with cold ethanol
20 and dried in a vacuum oven to give 3-(1H-indol-2-ylmethylene)-
4-methyl-1,3-dihydro-indol-2-one-

        ^{1}H NMR (360 MHz, DMSO-d6) δ 13.12 (s, br, 1H, NH), 11.02
(s, br, 1H, NH), 7.79 (s, 1H, H-vinyl), 7.64 (d, J = 8Hz, 1H),
7.57 (d, J = 8Hz, 1H), 7.27 (d, J = 7Hz, 1H), 7.23 (s, 1H),
25 7.05 - 7.14 (m, 2H), 6.84 (d, J = 8Hz, 1H), 6.78 (d, J = 8Hz,
1H), 2.61 (s, 3H, CH$_3$).

        MS El 274.

Example 167: <u>3-(3-Methyl-1H-indol-2-ylmethylene)-1,3-dihydro-</u>
<u>indol-2-one</u>

30   A mixture of 2-oxindole (133 mg), 3-methylindole-2-
carbaldehyde (190 mg) (prepared according to *Synthetic
Communications,* 1986, 16, 1799) and piperidine (10 mg) in
ethanol was held in a sealed tube at 95°C for 4 hours. The

mixture was cooled to room temperature. The solid was collected by vacuum filtration, washed with cold ethanol and dried in a vacuum oven to give 3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindole-2-one.

5   ^{1}H NMR (360 MHz, DMSO-d6) δ 13.01 (s, br, 1H, NH), 10.99 (s, br, 1H, NH), 7.89 (s, 1H, H-vinyl), 7.88 (d, J = 7Hz, 1H), 7.63 (d, J = 8Hz, 1H), 7.49 (d, J = 8Hz, 1H), 7.27 (dt, J = 1, 8Hz, 1H), 7.2 (dt, J = 1, 8Hz, 1H), 7.01-7.08 (m, 2H), 6.9 (d, J = 8Hz, 1H), 2.59 (s, 3H, CH$_3$).

10   MS El 274.

Example 168: **5-Chloro-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one**

A mixture of 5-chloro-2-oxindole (167 mg), 3-methylindole-2-carbaldehyde (190 mg) (prepared according to 15   *Synthetic Communications*, 1986, 16, 1799) and piperidine (10 mg) in ethanol was held in a sealed tube at 95°C for 4 hours. The mixture was cooled to room temperature. The solid was collected by vacuum filtration, washed with cold ethanol and dried in a vacuum oven to give 5-chloro-3-(3-methyl-1H-indol-20   2-ylmethylene)-1,3-dihydroindol-2-one.

^{1}H NMR (360 MHz, DMSO-d6) δ 12.96 (s, br, 1H, NH), 11.09 (s, br, 1H, NH), 8.09 (d, J = 2Hz, 1H), 8.02 (s, 1H, H-vinyl), 7.63 (d, J = 8Hz, 1H), 7.5 (d, J = 8Hz, 1H), 7.29 (dt, J = 1, 7Hz, 1H), 7.21 (dd, J = 2, 8Hz, 1H), 7.07 (dt, J = 1, 7Hz, 25   1H), 6.89 (d, J = 8Hz, 1H), 2.61 (s, 3H, CH$_3$).

MS El 308.

Example 169: **5-Bromo-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one**

A mixture of 5-bromo-2-oxindole (212 mg), 3-methyl-30   indole-2-carbaldehyde (190 mg) (prepared according to *Synthetic Communications*, 1986, 16, 1799) and piperidine (10 mg) in ethanol was held in a sealed tube at 95°C for 4 hours. The mixture was cooled to room temperature. The solid

was collected by vacuum filtration, washed with cold ethanol and dried in a vacuum oven to give 5-bromo-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one.

^{1}H NMR (360 MHz, DMSO-d6) δ 12.96 (s, br, 1H, NH), 11.09 (s, br, 1H, NH), 8.21 (d, J = 2Hz, 1H), 8.02 (s, 1H, H-vinyl), 7.63 (d, J = 8Hz, 1H), 7.5 (d, J = 8Hz, 1H), 7.33 (dd, J = 2, 8Hz, 1H), 7.29 (dt, J = 1, 7Hz, 1H), 7.07 (dt, J = 1, 7Hz, 1H), 6.84 (d, J = 8Hz, 1H), 2.62 (s, 3H, CH$_3$).

MS El 352 and 354.

Example 170: **4-Methyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one**

A mixture of 4-methyl-2-oxindole (147 mg), 3-methyl-indole-2-carbaldehyde (190 mg) (prepared according to *Synthetic Communications*, 1986, 16, 1799) and piperidine (10 mg) in ethanol was held in a sealed tube at 95°C for 4 hours. The mixture was cooled to room temperature. The solid was collected by vacuum filtration, washed with cold ethanol and dried in a vacuum oven to give 4-methyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one.

^{1}H NMR (360 MHz, DMSO-d6) δ 13.07 (s, br, 1H, NH), 11.0 (s, br, 1H, NH), 7.78 (s, 1H, H-vinyl), 7.64 (d, J = 8Hz, 1H), 7.51 (d, J = 8Hz, 1H), 7.27 (dt, J = 1, 8Hz, 1H), 7.04 - 7.12 (m, 2H), 6.85 (d, J = 8Hz, 1H), 6.78 (d, J = 8Hz, 1H), 2.64 (s, 3H, CH$_3$), 2.52 (s, 3H, CH$_3$).

MS El 288.

Example 171: **3-(1H-indol-2-ylmethylene)-5-[(1H-indol-2-ylmethylene)-amino]-1,3-dihydroindol-2-one**

A mixture of 5-amino-2-oxindole (74 mg), indole-2-carbaldehyde (87 mg) (prepared according to *Synthetic Communications*, 1983, 23, 3109) and piperidine (4 mg) in ethanol (2 mL) was held in a sealed tube at 95 °C for 3 hours. The mixture was cooled to room temperature. The solid was collected by vacuum filtration, washed with cold ethanol and

dried in a vacuum oven. The crude solid was then
chromatographed on silica gel to give 50 mg (25% yield) of
3-(1H-indol-2-ylmethylene)-5-[(1H-indol-2-ylmethylene)-
amino]-1,3-dihydro-indol-2-one

5   **Example 172:** __3-(1H-Indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-__
     __indole-5-sulfonic acid amide__

          ^{1}HNMR (300 MHz, DMSO-d6) δ 12.03 (br, s, 1H, NH), 10.80
     (br, s, 1H, NH), 9.37 (s, 1H), 8.09-8.24 (m, 3H), 7.51 (m,
     1H), 7.41 (m, 1H), 7.13 (m, 2H), 7.03 (s, 2H, NH_2), 6.85 (d, J

10   = 7.8 Hz, 1H).

          MS-EI m/z 339 [M]$^+$.

     Example 173: __3-(1H-Indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-__
     __indole-5-sulfonic acid methylamide__

          ^{1}HNMR (300 MHz, DMSO-d6) δ 12.75 (br, s, 1H, NH), 11.40

15   (br, s, 1H, NH), 8.15 (s, 1H, H-vinyl), 8.10 (d, J = 1.5 Hz,
     1H), 7.54-7.65 (m, 3H), 7.20-7.27 (m, 3H), 7.02-7.06 (m, 2H),
     2.38 (d, J = 4.8 Hz, 3H, CH_3).

          MS-EI m/z 353 [M]$^+$.

     Example 174: __3-(1H-Indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-__

20   __indole-5-carboxylic acid__

          ^{1}HNMR (300 MHz, DMSO-d6) δ 12.13 (br, s, 1H, NH), 10.88
     (br, s, 1H, NH), 8.22-8.50 (m, 2H), 7.66-7.94 (m, 2H), 7.52
     (m, 1H), 7.23 (m, 2H), 6.94 (m, 1H).

          MS-EI m/z 304 [M]$^+$.

25   **Example 175:** __3-(3-Methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-__
     __dihydro-1H-indole-5-sulfonic acid amide__

          ^{1}HNMR (360 MHz, DMSO-d6) δ 12.92 (br, s, 1H, NH), 11.35
     (br, s, 1H, NH), 8.36 (d, J = 2.2 Hz, 1H), 8.03 (s, 1H, H-
     vinyl), 7.67 (m, 2H), 7.53 (d, J = 7.6 Hz, 1H), 7.30 (m, 1H),

30   7.16 (s, 2H, NH_2), 7.08 (m, 1H), 7.03 (d, J = 8.6 Hz, 1H), 2.63
     (s, 3H, CH_3).

          MS-EI m/z 353 [M]$^+$.

     Example 176: __3-(1H-Indol-5-ylmethylene)-2-oxo-2,3-dihydro-1H-__

indole-5-sulfonic acid methylamide

    ^{1}HNMR (300 MHz, DMSO-d6) δ 11.38 (br, s, 1H, NH), 10.85 (br, s, 1H, NH), 8.11 (m, 1H), 8.01 (m, 1H), 7.90 (m, 1H), 7.78 (m, 1H), 7.37-7.53 (m, 3H), 7.10 (m, 1H), 6.95 (m, 1H),

5 6.4 (m, 1H), 2.28 (s, 3H, CH$_3$).

    MS-EI m/z 353 [M]$^+$.

Example 177:   <u>3-(1H-Indol-5-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid</u>

    ^{1}HNMR (300 MHz, DMSO-d6) δ 12.54 (br, s, 1H, NH), 10.86

10 (br, s, 1H, NH), 8.56, 7.76, 7.46, 6.91 (m, 9H).

Example 178:   <u>3-(1H-Indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid methylamide</u>

    ^{1}HNMR (300 MHz, DMSO-d6) δ 12.03 (br, s, 1H, NH), 10.84 (br, s, 1H, NH), 9.39 (s, 1H), 8.25 (s, 1H, H-vinyl), 8.22 (d,

15 J = 1.5 Hz, 1H), 8.16 (m, 1H), 7.40-7.46 (m, 2H), 7.06-7.15 (m, 2H), 7.04 (m, 1H, CH$_3$NH), 6.88 (d, J = 7.8 Hz, 1H), 2.32 (d, J = 5.1 Hz, 3H, CH$_3$).

    MS-EI m/z 355 [M]$^+$.

Example 179:   <u>5-Amino-3-(1H-indol-5-ylmethylene)-1,3-dihydro-indol-2-one</u>

20   MS-EI m/z 275 [M]$^+$.

Example 180:   <u>5-Amino-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one</u>

    ^{1}HNMR (300 MHz, DMSO-d6) δ 13.13 (br, s, 1H, NH), 10.57

25 (br, s, 1H, NH), 7.63 (s, 1H, H-vinyl), 7.61 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 7.03-7.06 (m, 2H), 6.61 (d, J = 8.3 Hz, 1H), 6.49 (dd, J = 1.4 & 8.3 Hz, 1H), 4.70 (br s, 2H, NH$_2$), 2.55 (s, 3H, CH$_3$).

    MS-EI m/z 289 [M]$^+$.

30 **Example 182:**   <u>3-(2-Methyl-1H-indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid</u>

    ^{1}HNMR (300 MHz, DMSO-d6) δ 12.0 (br, s, 1H, NH), 10.85 (br, s, 1H, NH), 7.90 (s, 1H, H-vinyl), 7.79 (dd, 1H), 7.41-

7.44 (m, 2H), 7.02-7.18 (m, 3H), 6.91 (d, 1H), 2.45 (s, 3H, CH$_3$).

MS-EI m/z 318 [M]$^+$.

Example 183:   3-(1H-Indol-5-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide

MS-EI m/z 367 [M]$^+$.

Example 184:   3-(2-Methyl-1H-indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide

^{1}HNMR (300 MHz, DMSO-d6) δ 11.95 (br, s, 1H, N*H*), 10.86 (br, s, 1H, N*H*), 7.84 (s, 1H, H-vinyl), 7.41 (dd, J = 1.8 & 8.4 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.04 (m, 1H), 6.85-6.95 (m, 4H).

MS-EI m/z 381 [M]$^+$.

Example 185:   3-(1H-Indol-5-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid amide

MS-EI m/z 339 [M]$^+$.

Example 186:   3-(1H-Indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide

^{1}HNMR (360 MHz, DMSO-d6) δ 12.80 (br, s, 1H, NH), 11.44 (br, s, 1H, NH), 8.30 (s, 1H, H-vinyl), 8.15 (d, J = 2.2 Hz, 1H), 7.70 (d, J = 7.2 Hz, 1H), 7.60 (m, 2H), 7.30 (m, 1H), 7.23 (s, 1H), 7.07-7.12 (m, 2H), 2.63 (s, 6H, 2xCH$_3$).

MS-EI m/z 367 [M]$^+$.

Example 187:   5-Amino-3-(2-methyl-1H-indol-3-ylmethylene)-1,3-dihydroindol-2-one

MS-EI m/z 289 [M]$^+$.

Example 188:   3-(1H-Indol-3-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide

^{1}HNMR (360 MHz, DMSO-d6) δ 12.11 (br, s, 1H, NH), 10.94 (br, s, 1H, NH), 9.50 (s, 1H), 8.44 (s, 1H), 8.33 (m, 2H), 7.51-7.53 (m, 2H), 7.25 (m, 2H), 7.03 (d, J = 7.9 Hz, 1H), 2.63 (s, 6H, 2xCH$_3$).

MS-EI m/z 367 [M]$^+$.

Example 189: <u>3-(2-Methyl-1H-indol-3-ylmethylene)-2-oxo-2,3-</u>
<u>dihydro-1H-indole-5-sulfonic acid amide</u>

MS-EI *m/z* 353 [M]⁺.

Example 190: <u>3-[2-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-</u>
5   <u>4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid</u>

1-(Morpholin-4-yl)cyclohexene (300 g), 214 g of
triethylamine and 1400 mL of dichloromethane were refluxed for
15 minutes and then cooled in a water bath to 15-20 °C. Ethyl
succinyl chloride (266 g) dissolved in 500 mL of
10   dichloromethane was added over 30 minutes. The mixture was
refluxed for 30 minutes and cooled to ambient temperature in a
water bath. The solid was collected by vacuum filtration,
washed with 100 mL of dichloromethane and discarded. The
filtrate was returned to the flask and the solvent removed by
15   distillation to give 454 g of crude 4-(2-morpholin-4-yl-
cyclohex-1-enyl)-4-oxo-butyric acid ethyl ester as an oil.

Crude 4-(2-morpholin-4-ylcyclohex-1-enyl)-4-oxo-butyric
acid ethyl ester (454 g), 398 g of diethyl aminomalonate
hydrochloride, 162 g of sodium acetate and 350 mL of glacial
20   acetic acid were heated to 108°C over 30 minutes. The mixture
was held at 100-108 °C for 2 hours and then cooled to about
50°C in a water bath. Water (2500 mL) and 700 mL of ethyl
acetate were added. The ethyl acetate layer was separated and
washed three times with brine, twice with saturated sodium
25   bicarbonate solutiona and once with brine. The solution was
dried over anhydrous sodium sulfate, and the ethyl acetate was
removed using a rotary evaporator to give 494 g (105% yield)
of crude 3-(2-ethoxycarbonylethyl)-4,5,6,7-tetrahydro-1H-
indole-2-carboxylic acid ethyl ester as an oil. The crude
30   mixture was chromatographed on a silica gel column using a
1:10 mixture of ethyl acetate:hexane as the eluent to give
122.1 g of pure 3-(2-ethoxycarbonylethyl)-4,5,6,7-tetrahydro-
1H-indole-2-carboxylic acid ethyl ester as a low melting

solid.

^{1}H NMR (d$_6$-DMSO): δ 11.0 (s, 1H, pyrrole NH), 4.2, 4.0 (t, each 4H, COCH$_2$), 2.8, 2.4 (t, each 4H, -CH$_2$CH$_2$CO-), 2.4 (m, 4H, -CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-).

Purified 3-(2-ethoxycarbonylethyl)-4,5,6,7-tetrahydro-1H-indole-2-carboxylic acid ethyl ester (122.1 g) and 328 mL of 5N sodium hydroxide were refluxed for 80 minutes. The heat was turned off and 165 mL of 10 N hydrochloric acid was carefully added to the vigorously stirred mixture using an addition funnel over the reflux condenser. Hydrochloric acid was added until the pH was 2-3. The mixture was then cooled in an ice bath at which time the oil in the mixture solidified. The solid was collected by vacuum filtration, washed 3 times with water and dried under vacuum at 50-60°C to give 54.9 g (71% yield) of 3-(4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid as a dark brown solid.

^{1}H NMR (d$_6$-DMSO): δ 13.1 (s, 1H, pyrrole NH), 11.8 (br s, 1H, COOH), 9.8 (s, 1H, CH), 2.5, 2.3 (t, 4H, -CH$_2$CH$_2$CO-), 2.4 (m, 4H, -CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-).

A mixture of 24 g of dimethylformamide and 300 mL of dichloromethane was cooled to -9°C. Phosphorus oxychloride (50 g) was rapidly added via an addition funnel. 3-(4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (54.9 g) was added in portions with vigorous stirring. The mixture was warmed to room temperature and then refluxed for 10 minutes. It was then cooled to 5 °C and diluted with 300 mL of water. The pH was adjusted to 10 using 10N sodium hydroxide. The layers were separated. The aqueous layer was cooled to 10°C and the pH adjusted to 2-3 with about 130 mL of 10 N hydrochloric acid. The oil which formed solidified and was collected by vacuum filtration, washed three times with water and dried under vacuum at 50°C to give 52.8 g (93% yield) of 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid as a

dark brown solid.

^{1}H NMR (d$_6$-DMSO): δ 13.1 (s, 1H, pyrrole NH), 11.7 (br s, 1H, COOH), 9.4 (s, 1H, CHO), 2.8, 2.5(t, 4H, -CH$_2$CH$_2$CO-), 2.4 (m, 4H, -CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-).

5   3-(2-Formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (5.4 g), 3.6 g of 2-oxindole and 2.7 g of piperidine (or 2.2 g of pyrrolidine) in 25 mL of ethanol were refluxed for 4 hours. Upon slow addition of acetic acid (8 mL), a precipitate formed. The mixture was refluxed for 5 minutes and cooled to

10   ambient temperature. The precipitate was collected by vacuum filtration and washed with 20 mL of ethanol. The solids were slurry-washed refluxing in 30 mL of ethanol, cooled, collected by vacuum filtration, washed with 30 mL of ethanol and dried under vacuum to give 5.5 g (68% yield) of 3-[2-(2-oxo-1,2-

15   dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid as an orange solid: mp 263-265 °C.

^{1}H NMR (d$_6$-DMSO): δ 13.1 (s, 1H, pyrrole NH), 12.0 (br s, 1H, COOH), 10.7 (s, 1H, CONH), 7.7, 7.1, 6.9, 6.8 (m, each 4H, aromatic), 7.6 (s, 1H, -CH=), 2.9, 2.7 (t, each 4H, -CH$_2$CH$_2$CO-),

20   2.4 (m, 4H, -CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-).

Example 191:   3-[2-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

       3-(2-Formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic

25   acid (5.4 g), 3.7 g of 5-chloro-2-oxindole and 2.7 g of piperidine (or 2.2 g of pyrrolidine) in 25 mL of ethanol was refluxed for 4 hours. Slow addition of acetic acid (8 mL) resulted in a precipitate. The mixture was refluxed for 5 minutes and cooled to ambient temperature. The precipitate

30   was collected by vacuum filtration and washed with 20 mL of ethanol. The solids were slurry-washed by refluxing in 30 mL of ethanol, cooled, collected by vacuum filtration, washed with 30 mL of ethanol and dried under high vacuum to give 6.5

g (80% yield) 3-[2-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1-H-indol-3-yl]-propionic acid as an orange solid: mp 370-384 °C.

^{1}H NMR (d_6-DMSO): δ 13.3 (s, 1H, pyrrole NH), 12.0 (br s,
5 1H, COOH), 10.7 (s, 1H, CONH), 7.8, 7.1, 6.8 (m, each 3H, aromatic), 7.7 (s, 1H, -CH=), 2.9, 2.7 (t, each 4H, CH$_2$CH$_2$CO), 2.5 (m, 4H, -CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-).

Example 192:   **3-[2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic**
10 **acid**

A mixture of 3-(2-Formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (3.4 g), 2.7 g of 5-bromo-2-oxindole and 1.4 g of pyrrolidine in 25 mL of ethanol was refluxed for 4 hours. Upon slow addition of acetic acid (8 mL), a
15 precipitate formed. The mixture was refluxed for 5 minutes and cooled to ambient temperature. The precipitate was collected by vacuum filtration and washed with 20 mL of ethanol. The solids were slurry-washed by refluxing in 30 mL of ethanol, cooled, collected by vacuum filtration, washed
20 with 30 mL of ethanol and dried under vacuum to give 5.2 g (98% yield) of 3-[2-(5-bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid as a red-orange solid, mp 286-289 °C.

^{1}H NMR (d_6-DMSO): δ 13.3 (s, 1H, pyrrole NH), 12.0 (br s,
25 1H, COOH), 10.7 (s, 1H, CONH), 7.9, 7.1, 6.8 (m, each 3H, aromatic), 7.7 (s, 1H, -CH=), 2.9, 2.7 (t, each 4H, -CH$_2$CH$_2$CO-), 2.5 (m, 4H, -CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-).

Example 193:   **3-[2-(4-Methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic**
30 **acid**

Diethyl oxalate (30 mL) in 20 mL of dry ether was added with stirring to 19 g of potassium ethoxide suspended in 50 mL of dry ether. The mixture was cooled in an ice bath and 20 mL

of 3-nitro-o-xylene in 20 mL of dry ether was slowly added.
The thick dark red mixture was refluxed for 0.5 hr,
concentrated, and treated with 10% sodium hydroxide until
almost all of the solid dissolved. Hydrogen peroxide (30%)

5   was then added to the dark red mixture until the red color
changed to yellow. The mixture was treated alternately with
10% sodium hydroxide and 30% hydrogen peroxide until the dark
red color was no longer present. The solid was filtered off
and the filtrate acidified with 6 N hydrochloric acid. The

10   resulting precipitate was collected by vacuum filtration,
washed with water, and dried under vacuum to give 9.8 g (45%
yield) of 1-methyl-6-nitrophenylacetic acid as an off-white
solid. The solid was hydrogenated in methanol over 10%
palladium on carbon to give 9.04 g of 4-methyl-2-oxindole as a

15   white solid.

        ^{1}H NMR (d$_6$-DMSO, 360 MHz) δ 10.27 (s, br, 1H, NH-1), 7.06
(t, 7.71 Hz, 1H, H-6), 6.74 (d, 7.73 Hz, H-5), 6.63 (d, 7.73
Hz, 1H, H-7), 3.36 (s, 2H, CH$_2$), 2.18 (s, 3H, CH$_3$).

        A mixture of 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-

20   yl)-propionic acid (5.4 g), 3.2 g of 4-methyl-2-oxindole and
2.7 g of piperidine in 25 mL of ethanol was refluxed for 4
hours. Upon slow addition of acetic acid (8 mL), a
precipitate formed. The mixture was refluxed for 5 minutes
and cooled to ambient temperature. The precipitate was

25   collected by vacuum filtration and washed with 20 mL of
ethanol. The solids were slurry-washed in 30 mL of refluxing
ethanol, cooled, collected by vacuum filtration, washed with
30 mL of ethanol and dried under vacuum to give 6.2 g (80%
yield) of 3-[2-(4-methyl-2-oxo-1,2-dihydroindol-3-

30   ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid as an orange solid.

        ^{1}H NMR (d$_6$-DMSO): δ 13.3 (s, 1H, pyrrole NH), 12.0 (br s,
1H, COOH), 10.7 (s, 1H, CONH), 7.7 (s, 1H, -CH=), 7.0, 6.8 (m,

each 2H, aromatic), 2.8, 2.7 (t, each 4H, -CH₂CH₂CO-), 2.6 (s, 1H, CH₃), 2.5 (m, 4H, -CH₂-, -CH₂-), 1.7 (m, 4H, -CH2CH2-).

Example 194: 3-[2-(5-Methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

A mixture of 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (5.4 g), 3.2 g of 5-methyl-2-oxindole and 2.7 g of piperidine in 25 mL of ethanol was refluxed for 4 hours. Upon addition of acetic acid (8 mL), a precipitate formed. The mixture was refluxed for 5 minutes and cooled to ambient temperature. The precipitate was collected by vacuum filtration and washed with 20 mL of ethanol. The solids were slurry-washed with 30 mL of refluxing ethanol, cooled, collected by vacuum filtration, washed with 30 mL of ethanol and vacuum dried to give 6.2 g (80% yield) of 3-[2-(5-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid as an orange solid.

¹H NMR (d₆-DMSO): δ 13.3 (s, 1H, pyrrole NH), 12.0 (br s, 1H, COOH), 10.7 (s, 1H, CONH), 7.7 (s, 1H, -CH=), 7.0, 6.8 (m, each 3H aromatic), 2.8, 2.7 (t, each 4H, -CH₂CH₂CO-), 2.6 (s, 1H, CH₃), 2.5 (m, 4H, -CH₂-, -CH₂-), 1.7 (m, 4H, -CH₂CH₂-).

MS: m/z 349.

Example 195: 3-[2-(6-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

A mixture of 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (5.4 g), 3.7 g of 6-chloro-2-oxindole and 2.7 g of piperidine in 25 mL of ethanol was refluxed for 4 hours. Upon slow addition of acetic acid (8 mL), a precipitate formed. The mixture was refluxed for 5 minutes and cooled to ambient temperature. The precipitate was collected by vacuum filtration and washed with 20 mL of ethanol. The solids were slurry-washed in 30 mL of reflusing

ethanol, cooled, collected by vacuum filtration, washed with
30 mL of ethanol and vacuum dried to give 6.5 g (80%) of 3-[2-
(6-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-
tetrahydro-1H-indol-3-yl]-propionic acid as an orange solid.

5   ^{1}H NMR (d$_6$-DMSO): δ 13.3 (s, 1H, pyrrole NH), 12.0 (br s,
1H, COOH), 10.7 (s, 1H, CONH), 7.7, 7.0, 6.9 (m, each 3H,
aromatic), 7.6 (s, 1H, -CH=), 2.9, 2.7 (t, each 4H, -CH$_2$CH$_2$CO-
), 2.4 (m, 4H, -CH$_2$-, -CH$_2$-), 1.7(m, 4H, -CH$_2$CH$_2$-).

       MS: m/z 371.

10   **Example 196:   3-[2-(6-Methoxy-2-oxo-1,2-dihydroindol-3-
     ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
     acid**

       A mixture of 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-
yl)-propionic acid (5.4 g), 3.6 g of 6-methoxy-2-oxindole and
15   2.7 g of piperidine in 25 mL of ethanol was refluxed for 4
hours. Upon slow addition of acetic acid (8 mL), a
precipitate formed. The mixture was refluxed for 5 minutes
and cooled to ambient temperature. The precipitate was
collected by vacuum filtration and washed with 20 mL of
20   ethanol. The solids were slurry-washed in 30 mL of reflusing
ethanol, cooled, collected by vacuum filtration, washed with
30 mL of ethanol and vacuum dried to give 6.4 g (80% yield) of
3-[2-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid as an orange
25   solid, mp 263-266 °C.

       ^{1}H NMR (d$_6$-DMSO): δ 13.0 (s, 1H, pyrrole NH), 12.0(s, 1H,
COOH), 10.7 (s, 1H, CONH), 7.6, 6.5, 6.4 (m, each 3H,
aromatic), 7.4 (s, 1H, -CH=), 3.7 (s, 3H, CH$_3$), 2.9, 2.7 (t,
each 4H, -CH$_2$CH$_2$CO-), 2.5 (m, 4H -CH$_2$-, -CH$_2$-), 1.7 (m, 4H,
30   -CH$_2$CH$_2$-).

       MS: m/z 365.

**Example 197:   N,N-Dimethyl-3-[2-(2-oxo-1,2-dihydroindol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide**

3-[2-(2-Oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-
tetrahydro-1H-indol-3-yl]-propionic acid (10 g) was dissolved
in 100 mL of dimethylformamide. Carbonyldiimidazole (6.3 g)
was added and the mixture stirred at ambient temperature for 1

5   hour. Dimethylamine (2.7 g) and 30 mL of dimethylformamide
were added and the stirring continued overnight at room
temperature. Fifty mL of water was added to the mixture and
stirring was continued for 10 minutes. The precipitate was
collected by vacuum filtration, washed with 20 mL of water and

10 then 20 mL of ethanol. The solid was slurry-washed in 30 mL
of refluxing ethanol for 5 minutes and cooled to room
temperature. The solid was collected by vacuum filtration,
washed with 20 mL of ethanol and vacuum dried to give 8.9 g
(83% yield) of N,N-dimethyl-3-[2-(2-oxo-1,2-dihydroindol-3-

15 ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide.

^{1}H NMR (d$_6$-DMSO): δ 13.1 (s, 1H, pyrrole NH), 10.7 (s, 1H,
CONH), 7.7, 7.1, 6.9, 6.8 (m, each 4H, aromatic), 7.6 (s, 1H,
-CH=), 3.3 (s, 6H, CH$_3$), 2.9, 2.7 (t, each 4H, -CH$_2$CH$_2$CO-), 2.5
(m, 4H -CH$_2$-, -CH$_2$-), 1.7(m, 4H, -CH$_2$CH$_2$-).

20   MS: m/z 364.

**Example 198:   3-[3-(3-Dimethylaminopropyl)-4,5,6,7-
tetrahydro-1H-indol-2-ylmethylene]-1,3-dihydroindol-2-one**

3-(4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (9.7
g) in 100 mL of tetrahydrofuran was stirred with 8.1 g of

25 carbonyl diimidazole for 1 hour. Dimethylamine (2.5 g) was
added and the mixture stirred for 2 hours. The solvent was
evaporated and the residue taken up in ethyl acetate, washed
with water, 0.1 N hydrochloric acid, water, and brine, dried
over sodium sulfate and evaporated to give 7.7 g (70% yield)

30 of 3-(4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid
dimethyl amide.

3-(4,5,6,7-Tetrahydro-1H-indol-3-yl)-propionic acid
dimethyl amide (7.7 g) and 13 g of borane-tetrahydrofuran

complex in 50 mL of tetrahydrofuran was refluxed for 3 hours.
The reaction was quenched with acetone and then water and
evaporated to dryness. The residue was chromatographed on
silica gel to give 2 g (20% yield) of 3-(3-dimethylamino-

5   propyl)-4,5,6,7-tetrahydro-1H-indole as a yellow oil.

Dimethylformamide (0.8 g) and 13 mL of dichloromethane
was cooled to -9 °C. Phosphorus oxychloride (1.7 g) was
rapidly added via a dropping funnel. 3-(3-dimethylamino-
propyl)-4,5,6,7-tetrahydro-1H-indole (2 g) was added in

10   portions with vigorous stirring. The mixture was warmed to
room temperature and then refluxed for 10 minutes. It was
then cooled to 5°C, and diluted with 20 mL of water. The pH
was adjusted to 10 with 10N sodium hydroxide. The layers were
separated. The organic layer was washed with water and brine,

15   dried over anhydrous sodium sulfate and evaporated to give 1.8
g (80%) of 3-(3-dimethylaminopropyl)-2-formyl-4,5,6,7-
tetrahydro-1H-indole as a dark oil which solidified.

3-(3-Dimethylaminopropyl)-2-formyl-4,5,6,7-tetrahydro-1H-
indole (1.8 g), 1 g of 2-oxindole and 0.1 g of piperidine in

20   10 mL of ethanol was refluxed for 4 hours and then cooled to
room temperature. The precipitate which formed was collected
by vacuum filtration and washed with 4 mL of ethanol. The
solids were slurry-washed with 8 mL of refluxing ethanol,
cooled, collected by vacuum filtration, washed with 3 mL of

25   ethanol and vacuum dried to give 1.8 g (70% yield) of 3-[3-(3-
dimethylaminopropyl)-4,5,6,7-tetrahydro-1H-indol-2-
ylmethylene]-1,3-dihydroindol-2-one as an orange solid.

^{1}H NMR (d$_6$-DMSO): δ 13.1 (s, 1H, pyrrole NH), 10.7 (s, 1H,
CONH), 7.6, 7.1, 7.0, 6.8 (m, each 4H, aromatic), 7.6 (s, 1H,

30   -CH=), 3.3 (s, 6H, CH$_3$), 2.7 (m, 4H, -CH$_2$-, -CH$_2$-), 2.5, 2.3,
1.4 (t, each 6H, -CH$_2$CH$_2$CH$_2$N-), 1.7 (m, 4H, -CH$_2$CH$_2$-).
MS: m/z 350.

Example 199:   3-[2-(2-Oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide

3-[2-(2-Oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid (10 g) was dissolved

5   in 100 mL of dimethylformamide. Carbonyldiimidazole (6.3 g) was added and the mixture stirred at ambient temperature for 1 hour. Ammonia (1 g) in 30 mL of dimethylformamide was added and the stirring continued overnight. Fifty mL of water was added to the mixture and stirring was continued for 10

10   minutes. The precipitate that formed was collected by vacuum filtration, washed with 20 mL of water and then 20 mL of ethanol. The solid was slurry-washed with 30 mL of refluxing ethanol for 5 minutes and cooled to room temperature. The solid was collected by vacuum filtration, washed with 20 mL of

15   ethanol and vacuum dried to give 11 g (83% yield) of 3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide.

^{1}H NMR (d$_6$-DMSO): δ 13.3 (s, 1H, pyrrole NH), 12.0 (br s, 1H, COOH), 10.7 (s, 1H, CONH), 7.6, 7.1, 6.9, 6.8 (m, each 4H,

20   aromatic), 7.6 (s, 1H, -CH=), 7.2, 6.7 (s, each 2H, NH$_2$), 2.9, 2.7 (t, 4H, -CH$_2$CH$_2$CO-), 2.4 (m, 4H, -CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-).

MS: m/z 336.

Example 200:   3-[3-(3-Morpholin-4-yl-3-oxopropyl)-4,5,6,7-

25   tetrahydro-1H-indol-2-ylmethylene]-1,3-dihydroindol-2-one

3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid (10 g) was dissolved in 100 mL of dimethylformamide. Carbonyldiimidazole (6.3 g) was added and the mixture stirred at ambient temperature for 1

30   hour. Morpholine (5.2 g) in 30 mL of dimethylformamide was added and the stirring continued overnight. Fifty mL of water was added to the mixture and stirring was continued for 10 minutes. The precipitate was collected by vacuum filtration,

washed with 20 mL of water and then 20 mL of ethanol. The solid that formed was slurry-washed with 30 mL of refluxing ethanol for 5 minutes and cooled to room temperature. The solid was collected by vacuum filtration, washed with 20 mL of ethanol and vacuum dried to give 9.6 g (80%) of 3-[3-(3-morpholin-4-yl-3-oxopropyl)-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene]-1,3-dihydroindol-2-one as an orange solid.

^{1}H NMR (d$_6$-DMSO): δ 13.3 (s, 1H, pyrrole NH), 12.0 (br s, 1H, COOH), 10.7 (s, 1H, CONH), 7.6, 7.1, 6.9, 6.8 (m, each 4H, aromatic), 7.6 (s, 1H, -CH=), 3.3 (multipets, 8H, morpholine CH$_2$), 2.9, 2.7 (t, each 4H, -CH$_2$CH$_2$CO-), 2.5 (m, 4H, CH$_2$, CH$_2$), 1.7 (m, 4H, -CH$_2$CH$_2$-).

MS: m/z 406.

Example 201:   N-Methyl-3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide

3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid (10 g) was dissolved in 100 mL of dimethylformamide. Carbonyldiimidazole (6.3 g) was added and the mixture stirred at ambient temperature for 1 hour. Methyl amine (1.8 g) in 30 mL of dimethylformamide was added and the stirring continued overnight. Fifty mL of water was added to the mixture and stirring was continued for 10 minutes. The precipitate was collected by vacuum filtration, washed with 20 mL of water and then 20 mL of ethanol. The solid was slurry-washed in 30 mL of refluxing ethanol for 5 minutes and cooled to room temperature. The solid was collected by vacuum filtration, washed with 20 mL of ethanol and vacuum dried to give 8.3 g (80% yield) of N-methyl-3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide as an orange solid.

MS: m/z 350.

Example 202:   N-(2-Morpholin-4-ylethyl)-3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-

Example 202:   N-(2-Morpholin-4-ylethyl)-3-[2-(2-oxo-1,2-
dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-
yl]-propionamide

5   3-[2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-
tetrahydro-1H-indol-3-yl]-propionic acid (10 g) was dissolved
in 100 mL of dimethylformamide. Carbonyldiimidazole (6.3 g)
was added and the mixture stirred at ambient temperature for 1
hour. 4-(2-Aminoethyl)morpholine (7.7 g) and 30 mL of

10   dimethylformamide were added and the stirring continued
overnight. Fifty mL of water was added to the mixture and
stirring was continued for 10 minutes. The precipitate was
collected by vacuum filtration, washed with 20 mL of water and
then 20 mL of ethanol. The solid was slurry-washed in 30 mL

15   of refluxing ethanol for 5 minutes and cooled to room
temperature. The solid was collected by vacuum filtration,
washed with 20 mL of ethanol and vacuum dried to give 1 g
(83%) of N-(2-morpholin-4-ylethyl)-3-[2-(2-oxo-1,2-dihydro-
indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-

20   propionamide, mp 256-258 °C.

^{1}H NMR (d$_6$-DMSO): δ 13.1 (s, 1H, pyrrole NH), 10.7 (s, 1H,
-CONH-), 7.7 (t, 1H, -CONHCH$_2$-), 7.6, 7.1, 6.9, 6.8 (m, each
4H, aromatic), 7.5 (s, 1H, -CH=), 3.5 (t, each 4H, -CH$_2$CH$_2$-),
3.1, 2.8 (m, each 2H, -CH$_2$NCH$_2$-), 2.7, 2.5 (t, each 4H, -

25   CH$_2$CH$_2$CO-), 2.3, 2.2 (m, each 4H, -NHCH$_2$CH$_2$N-), 2.2 (m, 4H, -
CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-).

Example 203:   3-[2-(2-Oxo-1,2-dihydropyrrolo[2,3-b]pyridin-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid

30   A mixture of 7-azaoxindole (99 mg), 110 mg of 3-(2-
formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid and 2
drops of piperidine in 2 mL of ethanol was refluxed for 5 h.
The reaction mixture was cooled and concentrated. The residue

was acidified with acetic acid to pH 6. The resulting
precipitate was collected by vacuum filtration, washed with
water and dried in a vacuum oven at 40 °C to give 25.4 mg (13%
yield) of 3-[2-2-oxo-1,2-dihydropyrrolo[2,3-b]pyridin-3-

5   ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid.

     MS: m/z 338.

Example 204:   3-{2-[6-(3-Methoxyphenyl)-2-oxo-1,2-dihydro-
indol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-

10   **propionic acid**

     A mixture of 103 mg 6-(3-methoxyphenyl)-2-oxindole, 95 mg
3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid
and piperidine (3 drops) in ethanol (2 mL) was heated in
sealed tube to 90°C and held there overnight. The reaction

15   mixture was concentrated and acidified with 6 N hydrochloric
acid. The precipitate that formed was collected by filtration
and washed with water and hexane to give 156 mg of 3-{2-[6-(3-
methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-
4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic acid as a brown

20   solid (82%).

     ^{1}H NMR (d$_6$-DMSO, 360 MHz): δ 13.26 (s, br, 1H, NH-1) 1.78
·(s, br, 1H, NH-1), 7.72 (d, 8.1 Hz, 1H, H-4), 7.65 (s, 1H, H-
vinyl), 7.35 (d, 7.9 Hz, 1H), 7.26 (dd, 1.3 Hz, 8.1 Hz, 1H, H-
5), 7.18 (d, 7.9 Hz 1H), 7.13 (t, 2.0 Hz, 1H), 7.09 (d, 1.3

25   Hz, 1H, H-7), 6.90 (dd,, 2.0 Hz, 1H), 3.82 (s, 3H, OCH$_3$), 2.91
(t, 7.4 Hz, 2H, CH$_2$CH$_2$COOH), 2.66 (t, 5.9 Hz, 2H, H-7'), 2.38-
2.46 (m, 4H, CH$_2$CH$_2$COOH and H-4'), 1.69-1.76 (m, 4H, H-5',6').

     MS: m/z 443.2.

Example 205:   3-{2-[6-(4-Methoxyphenyl)-2-oxo-1,2-dihydro-
30   indol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-
propionic acid

     A mixture of 103 mg 6-(4-methoxyphenyl)-2-oxindole, 95 mg
3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid

and piperidine (3 drops) in ethanol (2 mL) was heated to 90°C in a sealed tube and held there for 4 hrs. The reaction mixture was concentrated and acidified with 6 N hydrochloric acid. Ethyl acetate was added upon which a solid precipitated

5   from the aqueous layer. The precipitate was collected by filtration, and washed with water and hexane to give 57 mg of 3-{2-[6-(4-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic acid as a brown solid (30%).

10   ^{1}H NMR (d$_6$-DMSO, 360 MHz): δ 13.24 (s, br, 1H, NH-1), 11.61 (s, br, 1H, COOH), 10.76 (s, br, 1H, NH-1), 7. (d, 8.1 Hz, 1H, H-4), 7.61 (s, 1H, H-vinyl), 7.56 (d, 8.8 Hz, 2H, H-3, 5), 7.21 (dd, 1.5 Hz, 8.1 Hz, 1H, H-5), 7.04 (d, J = 1.5 Hz, 1H, H-7), 7.01 (d, 8.8 Hz, 2H, H-2″,6″), 3.79 (s, 3H, OCH$_3$),

15   2.91 (t, 7.4 Hz, 2H, CH$_2$CH$_2$COOH), 2.67 (t, 5.9 Hz, 2H, H-7′), 2.40-2.46 (m, 4H, CH$_2$CH$_2$COOH and H-4′), 1.72-1.78 (m, 4H, H-5′,6′).

        MS: m/z 441.2.

Example 206: <u>3-[2-(2-Oxo-6-phenyl-1,2-dihydroindol-3-</u>
20   <u>ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic</u>
<u>acid</u>

        A mixture of 90 mg 6-phenyl-2-oxindole, 95 mg 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid and piperidine (3 drops) in ethanol (2 mL) was held in a sealed

25   tube at 90 °C for 4 hrs. The reaction mixture was concentrated and acidified with 6 N hydrochloric acid. The precipitate was collected by filtration and washed with water and hexane to give 59 mg of 3-[2-(2-oxo-6-phenyl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-

30   propionic acid as a brown solid (31% yield).

        ^{1}H NMR (d$_6$-DMSO, 360 MHz): δ 13.27 (s, br, 1H, NH-1′), 12.06 (s, v br, 1H, COOH), 10.80 (s, br, 1H, NH-1), 7.74 (d, 7.9 Hz, 1H, H-4), 7.64 (s, 1H, H-vinyl), 7.62 (d, 7.7 Hz, 2H),

7.44 (t, 7.7 Hz, 2H), 7.32 (dd, 7.7 Hz, 1H), 7.27 (dd, 1.1,

7.9 Hz 1H, H-5), 7.10 (d, 1.1 Hz, 1H, H-7), 2.92 (t, 7.3 Hz,

2H, CH_2CH_2COOH), 2.67 (t, 5.5 Hz, 2H, H-7'), 2.41-2.46 (m, 4H,

CH_2CH_2COOH and H-4'), 1.73-1.76 (m, 4H, H-5',6').

5   MS: m/z 411.2.

Example 207:   3-{2-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydro-
indol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-
propionic acid

Tetrakis(triphenylphosphine)palladium (1 g) was added to

10   a mixture of 5 g of 2-methoxyphenylboronic acid, 6.6 g of 5-
bromo-2-fluoronitrobenzene and 30 mL of 2 M sodium carbonate

solution in 50 mL of toluene and 50 mL of ethanol. The

mixture was refluxed for 2 hours, concentrated, and the

residue extracted twice with ethyl acetate. The ethyl acetate

15   layer was washed with water and brine, dried, and concentrated

to give a dark green oil which solidified on standing to give

crude 4-fluoro-2'-methoxy-3-nitrobiphenyl.

Dimethyl malonate (14 mL) was added dropwise to 2.9 g of

sodium hydride suspended in 50 mL of dimethylsulfoxide. The

20   mixture was stirred at 100°C for 15 minutes and cooled to room

temperature. Crude 4-fluoro-2'-methoxy-3-nitrobiphenyl in 60

mL of dimethylsulfoxide was added and the mixture stirred at

100 °C for 2 hours. The reaction mixture was cooled, quenched

with 300 mL of saturated ammonium chloride solution and

25   extracted twice with ethyl acetate. The extracts were

combined, washed with saturated ammonium chloride, water, and

brine, dried over anhydrous sodium sulfate and concentrated to

give crude dimethyl 2'-methoxy-3-nitrobiphenyl-4-malonate as a

yellow oil.

30   Crude 2'-methoxy-3-nitrobiphenyl-4-malonate was stirred

at 100 °C in 50 mL of 6 N hydrochloric acid for 24 hours and

cooled. The precipitate that formed was collected by

filtration, washed with water and hexane, and dried to give

9.8 of 2'-methoxy-2-nitrobiphenyl-4-acetic acid as a light tan

solid.

Iron chips (5 g) were added in one portion to 9.8 g of
2'-methoxy-3-nitrobiphenyl-4-acetic acid in 50 mL of glacial
acetic acid and the mixture was stirred at 100 °C for 3 hours.
5   The reaction mixture was concentrated to dryness, sonicated
in ethyl acetate and filtered to remove insolubles. The
filtrate was washed twice with 1 N hydrochloric acid, then
with water and brine, dried over anhydrous sodium sulfate and
concentrated. The residue was chromatographed on silica gel
10 using ethyl acetate:hexane 1:2 as the eluent to give 5.4 g
(69% based on 5-bromo-2-fluoronitrobenzene) of 6-(2-
methoxyphenyl)-2-oxindole as a rose colored solid.

A mixture of 103 mg of 6-(2-methoxyphenyl)-2-oxindole, 95
mg of 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic
15 acid and piperidine (3 drops) in ethanol (2 mL) was held in a
sealed tube at 90 °C for 4 hrs. The reaction mixture was
concentrated and acidified with 6 N hydrochloric acid. The
precipitate was collected by filtration and washed with water
and hexane to give 67 mg of 3-{2-[6-(2-methoxyphenyl)-2-oxo-
20 1,2-dihydroindol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-
3-yl}-propionic acid as a brown solid (35% yield).

^{1}H NMR (d$_6$-DMSO, 360 MHz): δ 13.26 (s, br, 1H, H1, 20, vr
H OH) 10.71 (s, br, 1H, NH-1), 7.67 (d, 7.7 Hz, 1H, H-4), 7.61
(s, 1H, H-vinyl), 7.27-7.34 (m, 2H,), 7.01-7.10 (m, 2H), 7.05
25 (dd, 1.2, 7.7 Hz, 1H, H-5), 6.99 (d, 1.2 Hz, 1H, H-7), 3.75
(s, 3H, OCH$_3$), 2.91 (t, 7.5 Hz, 2H, CH$_2$CH$_2$COOH), 2.68 (t, 7 Hz,
2H, H-7'), 2.40-2.46 (m, 4H, CH$_2$CH$_2$COOH and H-4'), 1.71-1.78
(m, 4H, H-5',6').

MS m/z 441.2.

30 **Example 208:**   3-[2-(5-Isopropylaminosulfonyl-2-oxo-1,2-
dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-
yl]-propionic acid

To a 100 mL flask charged with 27 mL of chlorosulfonic

acid was slowly added 13.3 g of 2-oxindole. The reaction

temperature was maintained below 30 °C during the addition.

After the addition, the reaction mixture was stirred at room

temperature for 1.5 hr, heated to 68°C and stirred an

5   additional 1 hr, cooled, and poured into water. The

precipitate was washed with water and dried in a vacuum oven

to give 11.0 g of 5-chlorosulfonyl-2-oxindole (50% yield)

which was used without further purification.

A suspension of 3 g of 5-chlorosulfonyl-2-oxindole, 1.15

10   g of isopropylamine and 1.2 mL of pyridine in 50 mL of

dichloromethane was stirred at room temperature for 4 hours at

which time a white solid was present. The solid was collected

by vacuum filtration, slurry-washed with hot ethanol, cooled,

collected by vacuum filtration and vacuum dried at 40°C to

15   give 1.5 g (45%) of 5-isopropylaminosulfonyl-2-oxindole.

^{1}H NMR (d$_6$-DMSO, 300 MHz) δ 10.69 (s, br, 1H, NH), 7.63

(dd, 1.8 Hz, 1H, H-6), 7.59 (d, 1 Hz, 1H, H-4), 7.32 (d, 7 Hz,

1H, NH-SO$_2$-), 6.93 (d, 8 Hz, 1H, H-7), 3.57 (s, 2H, H-3), 3.14-

3.23 (m, 1H, CH-(CH$_3$)$_2$), 0.94 (d, 7 Hz, 6H, CH$_3$).

20   A mixture of (2-Formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-

propionic acid (5.4 g), 5.7 g of 5-isopropylaminosulfonyl-2-

oxindole and 2.7 g of piperidine in 25 mL of ethanol was

refluxed for 4 hours. Upon addition of acetic acid (8 mL), a

precipitate formed. The mixture was refluxed for 5 minutes

25   and cooled to ambient temperature. The precipitate was

collected by vacuum filtration and washed with 20 mL of

ethanol. The solids were slurry-washed in 30 mL of reflusing

ethanol, cooled, collected by vacuum filtration, washed with

30 mL of ethanol and vacuum dried to give 8.1 g (80% yield) of

30   3-[2-(5-isopropylaminosulfonyl-2-oxo-1,2-dihydroindol-3-

ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic

acid as an orange solid.

^{1}H NMR (d$_6$-DMSO): δ 13.3 (s, 1H, pyrrole NH), 12.0 (br s,

1H, COOH), 10.7 (s, 1H, CONH), 8.1, 7.5, 7.3, 7.0 (m, each 4H, aromatic), 7.7 (s, 1H, -CH=), 2.3 (m, 1H, -CH-), 2.9, 2.7 (t, each 4H, -CH$_2$CH$_2$CO-), 2.4 (m, 4H, -CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-), 0.9 (d, 6H, CH$_3$).

MS m/z 458.

Example 209:   3-[2-(6-Morpholin-4-yl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

A mixture of 4 g of 6-(morpholin-4-yl)-2-oxindole, 3.75 g of 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)propionic acid, and 1.8 mL of piperidine in ethanol (60 mL) was refluxed for 6 hrs. The reaction mixture was concentrated and acidified with 6 N hydrochloric acid to pH 6. The precipitate was collected by filtration, washed once with water, twice with ethyl acetate and twice with methanol to give 2.6 g of 3-[2-(6-Morpholin-4-yl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]propionic acid as an orange solid (34% yield).

^{1}H NMR (d$_6$-DMSO, 360 MHz): δ 13.04 (s, br, 1H, NH-1'), 12.05 (s, vbr, 1H, COOH), 10.60 (s, br, 1H, NH-1), 7.50 (d, 8.0 Hz, 1H, H-4), 7.39 (s, 1H, H-vinyl), 6.60 (d, 8.0 Hz, 1H, H-5), 6.43 (s, 1H, H-7), 3.73 (d, 4.7, 4H, H-2'', 6''), 3.09 (s, 4H, H-3'', 5''), 2.86 (t, 7.1 Hz, 2H, CH$_2$CH$_2$COOH), 2.64 (s, br, 2H, H-7'), 2.37-2.43 (m, 4H, CH$_2$CH$_2$COOH and H-4'), 1.71-1.75 (m, 4H, H-5',6').

MS m/z 422.3.

Example 210:   3-[2-(5-Chloro-4-methyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

A suspension of 3.0 g of 4-methyl-2-oxindole was stirred in 50 mL of acetonitrile at room temperature while 3.3 g of N-chlorosuccinimide was added in portions. Trifluoroacetic acid (1 mL) was then added. A white precipitate formed. The

suspension was stirred at room temperature for 3 days. The
white solid was collected by vacuum filtration, washed with a
small amount of cold acetone and dried overnight in a vacuum
oven at 40°C to give 2.5 g (68%) of 5-chloro-4-methyl-2-

5   oxindole.

A mixture of (2-Formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-
propionic acid (5.4 g), 4.0 g of 5-chloro-4-methyl-2-oxindole
and 2.7 g of piperidine in 25 mL of ethanol was refluxed for 4
hours. Addition of acetic acid (8 mL) resulted in the

10   formation of a precipitate. The mixture was refluxed for 5
minutes and cooled to ambient temperature. The precipitate
was collected by vacuum filtration and washed with 20 mL of
ethanol. The solids were slurry-washed with 30 mL of
refluxing ethanol, cooled, collected by vacuum filtration,

15   washed with 30 mL of ethanol and vacuum dried to give 6.8 g
(80%) of 3-[2-(5-Chloro-4-methyl-2-oxo-1,2-dihydroindol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid as an orange solid.

^{1}H NMR (d$_6$-DMSO): δ 13.1 (s, 1H, pyrrole NH), 12.0 (br s,

20   1H, COOH), 10.7 (s, 1H, CONH), 7.6 (s, 1H, -CH=), 7.3, 6.7(m,
each 2H, aromatic), 2.9, 2.7 (t, each 4H, -CH$_2$CH$_2$CO-), 2.7 (s,
3H, CH$_3$), 2.4(m, 4H, -CH$_2$-, -CH$_2$-), 1.7 (m, 4H, -CH$_2$CH$_2$-).

Example 211:   <u>3-[2-(5-Bromo-4-methyl-2-oxo-1,2-dihydroindol-
3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic</u>

25   <u>acid</u>

A mixture of (2-Formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-
propionic acid (5.4 g), 5.0 g of 5-bromo-4-methyl-2-oxindole
and 2.7 g of piperidine in 25 mL of ethanol was refluxed for 4
hours. Acetic acid (8 mL) was slowly added and a precipitate

30   formed. The mixture was refluxed for 5 minutes and cooled to
ambient temperature. The precipitate was collected by vacuum
filtration and washed with 20 mL of ethanol. The solids were
slurry-washed in 30 mL of refluxing ethanol, cooled, collected

by vacuum filtration, washed with 30 mL of ethanol and vacuum
dried to give 7.6 g (80%) of 3-[2-(5-bromo-4-methyl-2-oxo-1,2-
dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-
yl]-propionic acid as an red-orange solid.

5   ¹H NMR (d₆-DMSO): δ 13.1 (s, 1H, pyrrole NH), 12.0 (br s,
1H, COOH), 10.7 (s, 1H, CONH), 7.8 (s, 1H, -CH=), 7.3, 6.7,
(m, each 2H, aromatic), 2.9, 2.7 (t, each 4H, -CH₂CH₂CO-), 2.7
(s, 3H, CH₃), 2.4 (m, 4H -CH₂-, -CH₂-), 1.7 (m, 4H, -CH₂CH₂-).
MS: m/z 427,429.

10 **Example 212:**   <u>3-[2-(5-Bromo-2-oxo-1,2-dihydroindol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-N-(2-
morpholin-4-ylethyl)-propionamide</u>

        3-[2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid(12.3 g) was
15 dissolved in 150 mL of dimethylformamide. Carbonyldiimidazole
(6.3 g) was added and the mixture stirred at ambient
temperature for 1 hour. 4-(2-Aminoethyl)morpholine (7.7 g) and
30 mL of dimethylformamide were added and the stirring
continued overnight at room temperature. Fifty mL of water
20 was added to the mixture and stirring was continued for 10
minutes. The precipitate was collected by vacuum filtration,
washed with 20 mL of water and then 20 mL of ethanol. The
solid was slurry-washed in 30 mL of refluxing ethanol for 5
minutes and cooled to room temperature. The solid was
25 collected by vacuum filtration, washed with 20 mL of ethanol
and vacuum dried to give 12.5 g (80%) of 3-[2-(5-bromo-2-oxo-
1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-
3-yl]-N-(2-morpholin-4-ylethyl)-propionamide.

Example 213: <u>3-[2-(5-Chloro-2-oxo-1,2-dihydroindol-3-
30 ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-N-(2-
morpholin-4-ylethyl)-propionamide</u>

        3-[2-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid   (11 g) was

dissolved in 150 mL of dimethylformamide. Carbonyldiimidazole (6.3 g) was added and the mixture stirred at ambient temperature for 1 hour. 4-(2-Aminoethyl)morpholine (7.7 g) and 30 mL of dimethylformamide were added and the stirring

5   continued overnight at room temperature. Fifty mL of water was added to the mixture and stirring was continued for 10 minutes. The precipitate was collected by vacuum filtration, washed with 20 mL of water and then 20 mL of ethanol. The solid was slurry-washed in 30 mL of refluxing ethanol for 5

10   minutes and cooled to room temperature. The solid was collected by vacuum filtration, washed with 20 mL of ethanol and vacuum dried to give 11.5 g (80%) of 3-[2-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-N-(2-morpholin-4-ylethyl)-propionamide.

15   **Example 214:**   3-[2-(2-Oxo-1,2-dihydroindol-3-ylidenemethyl)-phenyl]-propionic acid

To a solution of 13.4 g of phthalic dicarboxaldehye in 100 mL of dichloromethane was added in portions over 5 minutes 35 g of ethyl (triphenyl- phosphoranylidene)acetate. The

20   reaction mixture was stirred at room temperature overnight and concentrated. The residue was stirred in 500 mL of a 6:1 mixture of hexanes:ethyl acetate for 1 hr. The solids were removed by fitration and the filtrates concentrated. The product was chromatographed on a silica gel column to give 10

25   g of ethyl 3-(2-formylphenyl)propenate as a mixture of E and Z isomers.

The above mixture was dissolved in 100 mL of ethyl acetate containing 100 mg of 5% palladium on carbon and stirred under hydrogen (balloon pressure) for 10 hrs. The

30   mixture was filtered through a bed of celite which was washed with ethyl acetate. The combined filtrates were concentrated to give 8 g of ethyl 3-(2-formylphenyl)propionate.

A mixture of 1 g of ethyl 3-(2-formylphenyl)propenate, 500 mg oxindole and 0.1 mL piperidine in 5 mL of ethanol was

stirred at 90 °C overnight. The mixture was evaporated and
purified on a silica gel column to give 450 mg of ethyl (E)-3-
[(2-oxo-1,2-dihydroindol-3-ylidenemethyl)phenyl]propionate
(M+1 = 322).

5   To 300 mg of ethyl (E)-3-[(2-oxo-1,2-dihydroindol-3-
ylidenemethyl)phenyl]-propionate in 3 mL of ethanol was added
2 mL of 2N sodium hydroxide. The mixture was stirred at 90°C
for 2 hrs, cooled and acidified with 6 N hydrochloric acid to
pH 3. The solid was collected by filtration and washed with
10   cold ethanol to give 30 mg of 3-[2-(2-oxo-1,2-dihydroindol-3-
ylidenemethyl)-phenyl]-propionic acid as a yellow solid.

^{1}H NMR (d$_6$-DMSO): δ 12.1 (s, 1H, COOH),10.6 (S, 1H, CONH),
7.7 (s, 1H, =CH) 7.6, 7.4, 7.3, 7.2, 7.0, 6.8 ,6.7 (m, 8H,
aromatic), 2.9, 2.5 (t, each 4H, CH$_2$CH$_2$).

15   MS: m/z 294.

**Example 215: 3-[4-Methyl-2-(2-oxo-1,2-dihydroindol-3-
ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid**

3,5-Dimethyl-4-(2-methoxycarbonylethyl)-1H-pyrrole-2-
carboxylic acid ethyl ester (127 g) was dissolved in acetic
20   acid (1900 mL), water (1900 mL) and tetrahydrofuran (1900 mL)
and cooled to -30 °C. Cerric ammonium nitrate (1097 g) was
added in portions with stirring to give a reddish-orange
suspension. The suspension was stirred at 0 °C for 2 hours,
neutralized to pH 7 with sodium bicarbonate and extracted with
25   ethyl acetate (2000 mL). The ethyl acetate layer was
separated, washed with brine (200 mL) and dried over anhydrous
sodium sulfate (20 g). The solvent was removed to give 80.2 g
(60%) of 5-formyl-4-(2-methoxycarbonylethyl)-3-methyl-1H-
pyrrole-2-carboxylic acid ethyl ester as an oil.

30   5-Formyl-4-(2-methoxycarbonylethyl)-3-methyl-1H-pyrrole-
2-carboxylic acid ethyl ester (80.2 g), 2-oxindole (37.9 g)
and ethanol (300 mL) were warmed to 70 °C in a 500 mL, 3-neck
round bottom flask equipped with mechanical stirring and a

reflux condenser. Piperidine (1.3 g) was added and the
mixture was refluxed for 4 hours. The mixture was cooled to
10°C and the orange precipitate collected by vacuum filtration
and washed with 30 mL of ethanol. The solid was slurry-washed
5   in 150 mL of refluxing ethanol, cooled, collected by vacuum
filtration, washed with 30 mL of ethanol and vacuum dried to
give 81.7 g (75%) of 4-(2-Methoxycarbonylethyl)-3-methyl-5-(2-
oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic
acid ethyl ester as an orange solid.

10   4-(2-Methoxycarbonylethyl)-3-methyl-5-(2-oxo-1,2-dihydro-
indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl
ester (81.7 g)), 56.5 g of potassium hydroxide, 200 mL of
ethanol and 200 mL of water were charged to a 1 L, 3 neck
round bottom flask equipped with mechanical stirring and a
15   thermometer. The mixture was stirred at 90°C for 90 minutes,
cooled to room temperature, and acidified with acetic acid
until a precipitate formed. The precipitate was collected by
vacuum filtration, washed with 50 mL of water and vacuum dried
to give 69.1 g (85 %) of 4-(2-carboxyethyl)-3-methyl-5-(2-oxo-
20   1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
as a red solid.

           4-(2-carboxyethyl)-3-methyl-5-(2-oxo-1,2-dihydroindol-3-
ylidenemethyl)-1H-pyrrole-2-carboxylic acid (10 g) suspended
in 50 mL of ethylene glycol (b.p. 198 °C) was sealed in a 1 L
25   pressure reactor, the reactor was pressurized to 120 psi with
nitrogen and then heated to 150 °C for 3 hours. The reaction
mixture was cooled to room temperature and then diluted with
50 mL of water. The resulting precipitate was collected by
vacuum filtration and was washed twice with 100 mL of water to
30   give a mixture of 3-[4-methyl-2-(2-oxo-1,2-dihydroindol-3-
ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid and 3-[4-methyl-
2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-
propionic acid 2-hydroxyethyl ester as a dark orange solid.

The solid was used in the next step without drying.

^{1}H NMR (d$_6$-DMSO) δ 13.3 (s, br, 1H, NH), 10.77 (s, 1 H, NH), 7.6 (s, 1 H, H-vinyl), 7.67, 7.08, 6.97, 6.85 (m, 4 H, Ar-H), 4.73 (t, J = 6Hz, 1 H, OH), 3.97-4.0 (m, 2H, CH$_2$), 3.5-3.55 (m, 2 H, CH$_2$), 2.98 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.51 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.04 (s, 3 H, CH$_3$).

MS m/z 341 (M+1).

3-[4-methyl-2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid and 3-[4-methyl-2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid 2-hydroxyethyl ester, 1.9 g of potassium hydroxide, 50 mL of water, and 50 mL of ethanol in a 500 mL 3 neck round bottom flask were stirred at 70°C for 1 hour. The mixture was cooled to room temperature and acidified with 2 N hydrochloric acid until a precipitate formed. The precipitate was collected by vacuum filtration and washed with ethanol:water mixture (1:1, 100mL). The solid was slurry-washed with ethyl acetate:ethanol mixture (1:1, 100 mL) at 70°C for 30 minutes and then cooled to room temperature. The product was collected by vacuum filtration and vacuum dried at 40°C overnight to give 7.8 g (90% overall yield) of 3-[4-methyl-2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid as a dark orange solid. (R. B. Woodward et al., *Tetrahedron*, **1990**, *46* *(22)*, 7599-7659)

^{1}HNMR (d$_6$-DMSO) δ 13.28 (s, br, 1 H, NH), 12.05 (s, 1 H, COOH), 10.78 (s, 1 H, NH), 7.68 (d, J = 7 Hz, 1 H, Ar-H), 7.64 (s, 1 H, H-vinyl), 7.11 (t, J = 7 Hz, 1 H, Ar-H), 7.11 (s, 1 H), 6.97 (t, J = 7 Hz, 1 H, Ar-H), 6.86 (d, J = 7 Hz, 1 H, Ar-H), 2.94 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.41 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.04 (s, 3H, CH$_3$).

Example 216:   3-[2-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid

5-Formyl-4-(2-ethoxycarbonylethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester (281 mg), 5-chloro-2-oxindole (168 mg), and piperidine (2 drops) in ethanol (2 mL) were refluxed for 2 hours. The reaction mixture was cooled and

5   the precipitate that formed was filtered, washed with ethanol and hexanes, and dried to give 369 mg (86%) of 4-(2-ethoxycarbonylethyl)-3-methyl-5-(5-chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester as a light yellow needle crystals.

10   A mixture of 4-(2-ethoxycarbonylethyl)-3-methyl-5-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester (346 mg) and potassium hydroxide (560 mg) in ethanol (5 mL) was heated to 95°C and then cooled, upon which red crystals formed. The crystals were

15   dissolved in water and acidified with 2 N hydrochloric acid to pH 2. The precipitate was filtered, washed with water, and dried in a vacuum oven overnight to give 299 mg (100%) of 4-(2-carboxyethyl)-3-methyl-5-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid

20   as a brown solid.

4-(2-carboxyethyl)-3-methyl-5-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid suspended in ethylene glycol (5 mL) was heated in a sealed tube in a pre-heated oil bath at 200 °C for 2 hours. The

25   reaction mixture was cooled to 90 °C and potassium hydroxide (2 pellets) were added. The mixture was then heated at 90°C for 30 minutes, after which time it was cooled, poured into water, and acidified with 2 N hydrochloric acid to pH 2. The precipitate that formed was filtered, washed with water,

30   and dried in a vacuum oven overnight to give 77 mg (29%) of 3-[2-(5-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid.

^{1}HNMR (d$_6$-DMSO) δ 13.31 (s, br. 1 H, NH), 12.05 (s, 1 H,

COOH), 10.89(s, br, 1 H, NH), 7.85 (d, J = 2 Hz, 1 H, H-4),

7.75 (s, 1 H, H-vinyl), 7.16 (d, J = 3 Hz, 1 H), 7.11 (dd, J

= 2.8 Hz, 1 H, H-6), 6.84 (d, J = 8 Hz, 1 H, H-7), 2.97 (t,

J = 7.5 Hz, 2 H, CH$_2$), 2.41 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.04

5   (s, 3 H, CH$_3$).

MS m/z 331 (M+1).

Example 217:   3-[2-(6-Methoxy-2-oxo-1,2-dihydroindol-3-
ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid

5-Formyl-4-(2-ethoxycarbonylethyl)-3-methyl-1H-pyrrole-

10   2-carboxylic acid ethyl ester (591 mg), 6-methoxy-2-oxindole

(333 mg) and piperidine (0.1 mL) in ethanol (4 mL) were

stirred at 90°C for 2 hours. Potassium hydroxide (537 mg)

was added to the mixture and it was then stirred at 95°C for

and additional hour. The reaction mixture was cooled and

15   concentrated, dissolved in water, and acidified with 2 N

hydrochloric acid to pH 2. The precipitate was filtered,

washed with water, and dried in a vacuum oven overnight to

give 730 mg (99%) of 4-(2-carboxyethyl)-3-methyl-5-(6-

methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-

20   2-carboxylic acid.

4-(2-carboxyethyl)-3-methyl-5-(6-methoxy-2-oxo-1,2-

dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid

(501 mg) suspended in ethylene glycol (10 mL) was heated in

a sealed tube in a pre-heated oil bath at 200°C for 2 hours.

25   The reaction mixture was cooled to 90 °C and potassium

hydroxide (2 pellets) was added. The mixture was then

stirred at 90°C for 30 minutes, after which time it was

cooled, poured into water, and acidified with 2 N

hydrochloric acid to pH 2. The precipitate was filtered,

30   washed with water, and dried in a vacuum oven overnight to

give 221 mg (48%) of 3-[2-(6-methoxy-2-oxo-1,2-dihydro-

indol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic

acid.

^{1}HNMR (d$_{6}$-DMSO) δ 13.09 (s, 1 H, NH), 12.05 (s, 1 H, COOH), 10.74 (s, 1 H, NH), 7.58 (d, *J* = 8 Hz, 1 H, H-4), 7.48 (s, 1 H, H-vinyl), 7.04 (d, *J* = 2 Hz, 1 H), 6.56 (dd, *J* = 2.8 Hz, 1 H, H-5), 6.43 (d, *J* = 2 Hz, 1 H), 3.75 (s, 3 H, OCH$_{3}$), 2.91 (t, *J* = 7.5 Hz, 2 H, CH$_{2}$), 2.4 (t, *J* = 7.5 Hz, 2 H, CH$_{2}$), 2.03 (s, 3 H, CH$_{3}$).

MS m/z 325 (M-1).

Example 218: <u>3-[2-(4-Methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid</u>

A mixture of 5-formyl-4-(2-ethoxycarbonylethyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (281 mg), 4-methyl-2-oxindole (147 mg) and piperidine (2 drops) in ethanol (2 mL) was stirred at 90°C for 2 hours. Potassium hydroxide (213 mg) was added, the temperature was increased to 95°C and held there for 1 hour. The reaction mixture was cooled and concentrated. The residue was dissolved into water and acidified with 2 N hydrochloric acid to pH 2. The precipitate was filtered, washed with water and dried in a vacuum oven overnight to give 337 mg (95%) of 4-(2-carboxyethyl)-3-methyl-5-(4-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid.

4-(2-carboxyethyl)-3-methyl-5-(4-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-2-carboxylic acid (300 mg) suspended in ethylene glycol (5 mL) was heated in a sealed tube in a pre-heated oil bath at 200 °C for 2 hours. The reaction mixture was cooled to 90°C and potassium hydroxide (1 pellet) was added. It was then stirred at 90°C for 30 minutes. The reaction mixture was cooled, poured into water, and acidified with 2 N hydrochloric acid to pH 2. The precipitate was filtered, washed with water, and purified on a silica gel column to give 115 mg (44% yield) of 3-[2-(4-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid.

^{1}HNMR (d$_{6}$-DMSO) δ 13.37 (s, 1 H, NH), 12.04 (s, 1 H, COOH), 10.81 (s, 1 H, NH), 7.69 (s, 1 H, H-vinyl), 7.09 (d, J = 2.5 Hz, 1 H), 7.01 (t, J = 7.5 HZ, 1 H, Ar-H), 6.79 (d, J = 7.5 Hz, 1 H, Ar-H), 6.74 (d, J = 7.5 Hz, 1 H, Ar-H), 2.88 (t,

5   J = 7.2 Hz, 2 H, CH$_{2}$), 2.61 (s, 3 H, CH$_{3}$-4), 2.44 (t, J = 7.2 Hz, 2 H, CH$_{2}$), 2.04 (s, 3 H, CH$_{3}$).

MS m/z 309 (M-1).

Example 219: <u>3-[2-(6-Chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid</u>

10   5-Formyl-4-(2-ethoxycarbonylethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester (281 mg), 6-chloro-2-oxindole (168 mg) and piperidine (2 drops) in ethanol (2 mL) were stirred at 90 °C for 2 hours. Potassium hydroxide (537 mg) was added to the mixture and it was then stirred at 95°C for

15   and additional hour. The reaction mixture was cooled and concentrated. The residue was dissolved into water and acidified with 2 N hydrochloric acid to pH 2. The precipitate was filtered, washed with water, and dried in a vacuum oven overnight to give 270 mg (72%) of 4-(2-carboxy-

20   ethyl)-3-methyl-5-(6-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid.

        4-(2-carboxyethyl)-3-methyl-5-(6-chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (240 mg) suspended in ethylene glycol (5 mL) was held in a

25   sealed tube in a pre-heated oil bath at 200 °C for 2 hours. The reaction mixture was cooled to 90°C and potassium hydroxide (2 pellets) was added. It was then stirred at 90°C for 30 minutes. The reaction mixture was cooled, poured into water, and acidified with 2 N hydrochloric acid to pH 2. The

30   precipitate was filtered, washed with water, and purified on a silica gel column using ethyl acetate:hexanes:glacial acetic acid 50:50:10 as eluent to give 45 mg (21%) of 3-[2-(6-chloro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-

yl]-propionic acid.

^{1}HNMR (d$_6$-DMSO) δ 13.2 (s, 1H, NH), 12.04 (s, 1 H, COOH), 10.92 (s, 1 H, NH), 7.72 (d, J = 8 Hz, 1 H, H-4), 7.68 (s, 1 H, H-vinyl), 7.15 (d, J = 2.4 HZ, 1 H), 7.01 (dd, J = 2.8 Hz, 1 H, H-6), 6.86 (d, J = 2 Hz, 1 H, H-7), 2.94 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.4 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.03 (s, 3 H, CH$_3$).

MS m/z 329 (M-1).

Example 220:   3-[2-(5-Bromo-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid

A mixture of 5-formyl-4-(2-ethoxycarbonylethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester (281 mg), 5-bromo-2-oxindole (220 mg), and piperidine (2 drops) in ethanol (2 mL) was stirred at 90 °C for 2 hours. Potassium hydroxide (537 mg) was added and the temperature was increased to 95° C for 1 hour. The reaction mixture was cooled and concentrated. The residue was dissolved into water and acidified with 2 N hydrochloric acid to pH 2. The precipitate that formed was filtered, washed with water and dried in a vacuum oven overnight to give 411 mg (98%) of 4-(2-carboxyethyl)-3-methyl-5-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid.

4-(2-carboxyethyl)-3-methyl-5-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (380 mg) suspended in ethylene glycol (5 mL) was held in a sealed tube in a pre-heated oil bath at 200°C for 2 hours. The reaction mixture was cooled to 90°C and potassium hydroxide (1 pellet) was added. It was then stirred at 90°C for 30 minutes. The reaction mixture was cooled, poured into water, and acidified with 2 N hydrochloric acid to pH 2. The precipitate was filtered, washed with water, and purified using silica gel column chromatography to give 168 mg (49% yield) of 3-[2-(5-bromo -2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid.

^{1}HNMR (d$_6$-DMSO) δ 13.32 (s, 1 H, NH), 12.0 (s, 1 H, COOH), 10.9 (s, 1 H, NH), 7.97 (d, J = 2 HZ, 1 H, h-4), 7.75 (s, 1 H, H-vinyl), 7.23 (dd, v = 2.8 Hz, 1 H, H-6), 7.16 (d, v = 2.6 Hz, 1 H), 6.8 (d, J = 8 Hz, 1 H, H-7), 2.97 (t, J = 7.7 Hz, 2 H, CH$_2$), 2.41 (t, J = 7.7 Hz, 2 H, CH$_2$), 2.04 (s, 3 H, CH$_3$).

MS m/z 375/377.

Example 221:   **3-[2-(5-Methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid**

5-Methylisatin (15.0 g) and 60 mL of hydrazine hydrate were heated to 140-160°C for 4 hours. Thin layer chromatography (ethyl acetate:hexane 1:2, silica gel) showed no starting material remaining. The reaction mixture was cooled to room temperature, poured into 300 mL of ice water, and acidified to pH 2 with 6 N hydrochloric acid. After standing at room temperature for 2 days, the precipitate was collected by vacuum filtration, washed with water, and dried under vacuum to give 6.5 g (47%) of 5-methyl-2-oxindole.

^{1}HNMR (360 MHz, DMSO-d$_6$) δ 10.20 (s, br, 1 H, NH-1), 6.99 (s, 1 H, H-4), 6.94 (d, J = 8.11 Hz, 1 H, H-6), 6.68 (d, J = 8.11 Hz, 1 H, H-7), 3.39 (s, 2 H, CH$_2$-3), and 2.22 (s, 3 H, CH$_3$-5).

5-Formyl-4-(2-ethoxycarbonylethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester (560 mg), 5-methyl-2-oxindole (300 mg), and piperidine (4 drops) in ethanol (4 mL) were stirred at 90°C for 2 hours. Potassium hydroxide (537 mg) was added to the mixture and it was then stirred at 95°C for an additional hour. The reaction mixture was cooled and concentrated. The residue was dissolved into water and acidified with 2 N hydrochloric acid to pH 2. The precipitate was filtered, washed with water, and dried in a vacuum oven overnight to give 496 mg of 4-(2-carboxyethyl)-3-methyl-5-(5-methyl -2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid.

4-(2-carboxyethyl)-3-methyl-5-(5-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid (496 mg) suspended in ethylene glycol (2 mL) was held in a sealed tube in a pre-heated oil bath at 200°C for 2 hours.

5   The reaction mixture was cooled to 90°C and potassium hydroxide (157 mg) was added. It was then stirred at 90°C for 30 minutes. The reaction mixture was cooled, poured into water, and acidified with 2 N hydrochloric acid to pH 2. The precipitate was filtered, washed with water, and dried in a

10 vacuum oven overnight to give 128 mg (29%) of 3-[2-(5-methyl-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid.

^{1}HNMR (d$_6$-DMSO) δ 13.31 (s, 1 H, NH), 12.07 (s, 1 H, COOH), 10.68 (s, 1 H, NH), 7.59 (s, 1 H), 7.51 (br s, 1 H, H-

15 4), 7.09 (d, J = 2.7 Hz, 1H), 6.91 (br d, J = 8 HZ, 1 H, H-6), 6.73 (d, J = 8 Hz, 1 H, H-7), 2.93 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.41 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.3 (s, CH$_3$-5), 2.04 (s, 3 H, CH$_3$).

MS m/z 311 (M+1).

20 **Example 222:   3-[2-(5-Methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-pyrrol-3-yl]-propionic acid**

A mixture of 5-formyl-4-(2-ethoxycarbonylethyl)-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester (562 mg), 5-methoxy-2-oxindole (326 mg), and piperidine (2 drops) in

25 ethanol (2 mL) were stirred at 90° C for 2 hours. Potassium hydroxide (537 mg) was added, the temperature was increased to 95°C and held there for 1 hour. The reaction mixture was cooled and concentrated. The residue was dissolved into water and acidified with 2 N hydrochloric acid to pH 2. The

30 precipitate was filtered, washed with water, and dried in a vacuum oven overnight to give 240 mg (65%) of 4-(2-carboxyethyl)-3-methyl-5-(5-methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid.

4-(2-carboxyethyl)-3-methyl-5-(5-methoxy-2-oxo-1,2-
dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
(240 mg) suspended in ethylene glycol (2 mL) was held in a
sealed tube in a pre-heated oil bath at 200°C for 2 hours.

5   The reaction mixture was cooled to 90°C and potassium
hydroxide (1 pellet) was added. It was then stirred at 90°C
for 30 minutes. The reaction mixture was cooled, poured into
water and acidified with 2 N hydrochloric acid to pH 2. The
precipitate was filtered, washed with water, and dried in a

10   vacuum oven overnight to give 30.5 mg (14%) of 3-[2-(5-
methoxy-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-4-methyl-1H-
pyrrol-3-yl]-propionic acid.

^{1}HNMR (d$_6$-DMSO) δ 13.38 (s, 1 H, NH), 12.07 (s, 1 H,
COOH), 10.59 (s, 1 H, NH), 7.63 (s, 1 H, H-vinyl), 7.1 (d, J =

15   2.1 Hz, 1 H), 6.75 (d, J = 8 Hz, 1 H, H-7), 6.69 (dd, J = 2.8
Hz, 1 H, H-6), 3.76 (s, 3 H, OCH$_3$), 2.96 (t, J = 7.4 Hz, 2 H,
CH$_2$), 2.41 (t, J = 7.4 Hz, 2 H, CH$_2$), 2.04 (s, 3 H, CH$_3$).

MS m/z 327 (M+1).

Example 223:   **3-{2-[6-(3-Methoxyphenyl)-2-oxo-1,2-dihydro-**

20   **indol-3-ylidenemethyl]-4-methyl-1H-pyrrol-3-yl}-propionic acid**

Tetrakis(triphenylphosphine)palladium (0.7 g) was added
to a mixture of 5 g of 3-methoxyphenylboronic acid, 3.8 g of
5-bromo-2-fluoronitrobenzene, and 11 mL of 2 M sodium
carbonate solution in 100 mL of toluene. The mixture was

25   refluxed for 2 hours, diluted with water and extracted with
ethyl acetate. The ethyl acetate was washed with saturated
sodium bicarbonate and brine, dried, and concentrated to give
an oily solid. The solid was purified on a silica gel column
using a 1:6 mixture of ethyl acetate:hexane as eluent, to give

30   4.3 g (77%) of 4-fluoro-3'-methoxy-3-nitrobiphenyl.

Dimethyl malonate (9.7 mL) was added dropwise to 2.0 g of
sodium hydride suspended in 50 mL of dimethylsulfoxide. The
mixture was stirred at 100°C for 35 minutes and cooled to room

temperature. 4-Fluoro-2'-methoxy-3-nitrobiphenyl (4.2 g) in
50 mL of dimethylsulfoxide was added and the mixture was
stirred at 100°C for 1 hour. The reaction mixture was cooled,
quenched with 300 mL of saturated ammonium chloride solution

5   and extracted twice with ethyl acetate. The extracts were
combined, washed with brine, dried over anhydrous sodium
sulfate and concentrated to give crude dimethyl 3'-methoxy-3-
nitrobiphenyl-4-malonate as a pale yellow solid.

     Crude 3'-methoxy-3-nitro-biphenyl4-malonate was stirred

10   at 110°C in 45 mL of 6 N hydrochloric acid for 4 days and then
cooled. The precipitate which formed was collected by
filtration, washed with water and hexane, and dried to give
5.3 g of 3'-methoxy-2-nitrobiphenyl-4-acetic acid as a light
tan solid.

15   3'-Methoxy-3-nitrobiphenyl-4-acetic acid (5.2 g) was
dissolved in methanol and hydrogenated over 0.8 g of 10%
palladium on carbon for 3 hours at room temperature. The
catalyst was removed by filtration, washed with methanol,
and the filtrates combined and concentrated to give a brown

20   solid. The solid was purified on a silica gel column, using
a 33:66:1 mixture of ethyl acetate:hexane:acetic acid as
eluent, to give 3.0 g (75 % based on 4-fluoro-3'-methoxy-3-
nitrobiphenyl) of 6-(3-methoxypheny)-2-oxindole as a pink
solid.

25   ^{1}HNMR (360 MHz, DMSO-d$_6$) δ 10.39 (s, br, 1 H, NH), 7.35
(t, J = 7.85 Hz, 1 H), 7.26 (d, J = 7.78 Hz, 1 H), 7.19 (dd,
J = 1.22, 7.8 Hz, 1 H), 7.13-7.16 (m, 1 H), 7.09-7.1 (m, 1
H), 7.01 (d, J = 1.48 Hz, 1 H), 6.90-6.93 (m, 1 H), 3.8 (s,
3 H, OCH$_3$), 3.49 (s, 2H, CH$_2$).

30   MS m/z (relative intensity, %) 240.0 ([M+1]$^+$, 100).

     5-Formyl-4-(2-ethoxycarbonylethyl)-3-methyl-1H-pyrrole-
2-carboxylic acid ethyl ester (281 mg), 6-(3-methoxy-
phenyl)-2-oxindole (287 mg), and piperidine (2 drops) in

ethanol (5 mL) were stirred at 90°C overnight. The
precipitate was filtered and washed with ethanol. The
yellow orange solid and potassium hydroxide (4 pellets) were
stirred in ethanol (3 mL) at 90°C for 2.5 hours. The

5   reaction mixture was cooled and concentrated. The residue
was dissolved into water and acidified with 2 N hydrochloric
acid to pH 2. The precipitate was filtered, washed with
water, and dried in a vacuum oven overnight to give 413 mg
of 4-(2-Carboxyethyl)-5-[6-(3-methoxyphenyl)-2-oxo-1,2-

10 dihydroindol-3-ylidenemethyl]-3-methyl-1H-pyrrole-2-
carboxylic acid.

        4-(2-Carboxyethyl)-5-[6-(3-methoxyphenyl)-2-oxo-1,2-
dihydroindol-3-ylidenemethyl]-3-methyl-1H-pyrrole-2-carboxylic
acid (413 mg) suspended in ethylene glycol (5 mL) was held in

15 ealed tube in a pre-heated oil bath at 200°C for 2 ½ hours.
The reaction mixture was cooled to 90°C and potassium
hydroxide (4 pellets) was added. It was then stirred at 100°C
for 2 more hours. The reaction mixture was cooled, poured
into water and acidified with 2 N hydrochloric acid to pH 2.

20 The precipitate was filtered, washed with water and dried in a
vacuum oven overnight. The crude solid was purified on a
silica gel column using 33:66:1 ethyl acetate:hexanes:glacial
acetic acid as eluent to give 75 mg (20%) of 3-{2-[6-(3-
methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4-

25 methyl-1H-pyrrol-3-yl}-propionic acid as an orange-red solid.

        ^{1}HNMR (d$_6$-DMSO) δ 13.27 (s, 1 H, NH), 12.06 (s, 1 H,
COOH), 10.86 (s, 1 H, NH), 7.76 (d, J = 8 Hz, 1 H, H-4), 7.68
(s, 1 H, H-vinyl), 7.36 (t, J = 8 Hz, 1 H), 7.29 (dd, J = 1.5,
8 Hz, 1 H), 6.92, 7.09, 7.13,7.2 (m, 5 H, Ar-H), 3.82 (s, 3 H,

30 OCH$_3$), 2.96 (t, J = 7.4 Hz, 2 H, CH$_2$), 2.42 (t, J = 7.4 Hz, 2
H, CH$_2$), 1.97 (s, 3H, CH$_3$).

Example 224:   3-{2-[6-(3-Ethoxyphenyl)-2-oxo-1,2-dihydro-
indol-3-ylidenemethyl]-4-methyl-1H-pyrrol-3-yl}-propionic acid

A mixture of 5-formyl-4-(2-ethoxycarbonylethyl)-3-
methyl-1H-pyrrole-2-carboxylic acid ethyl ester (281 mg), 6-

5 (3-ethoxyphenyl)-2-oxindole (304 mg), and piperidine (2

drops) in ethanol (5 mL) were held at 90 °C overnight. The

precipitate that formed was filtered, washed with ethanol.

The precipitate, an orange solid, was stirred with potassium

hydroxide (4 pellets) in ethanol (3 mL) at 90°C for 2.5

10 hours. The reaction mixture was cooled and concentrated.

The residue was dissolved into water and acidified with 2N

hydrochloric acid to pH 2. The precipitate was filtered,

washed with water, and dried in a vacuum oven overnight to

give 370 mg of 4-(2-Carboxyethyl)-5-[6-(3-ethoxyphenyl)-2-

15 oxo-1,2-dihydroindol-3-ylidenemethyl]-3-methyl-1H-pyrrole-2-

carboxylic acid.

4-(2-Carboxyethyl)-5-[6-(3-ethoxyphenyl)-2-oxo-1,2-

dihydroindol-3-ylidenemethyl]-3-methyl-1H-pyrrole-2-carboxylic

acid (350 mg) suspended in ethylene glycol (5 mL) was held in

20 a sealed tube in a pre-heated oil bath at 200°C for 2.5 hours.

The reaction mixture was cooled to 100°C and potassium

hydroxide (4 pellets) was added. It was then stirred at 100°C

for 2 hours. The reaction mixture was cooled, poured into

water, and acidified with 2 N hydrochloric acid to pH 2. The

25 precipitate was filtered, washed with water, and dried in a

vacuum oven overnight. The crude solid was purified on a

silica gel column using 33:66:1 ethyl acetate:hexanes:glacial

acetic acid to give 140 mg (44%) of 3-{2-[6-(3-ethoxyphenyl)-

2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4-methyl-1H-pyrrol-3-

30 yl}-propionic acid as a brown solid.

^{1}HNMR (d$_6$-DMSO) δ 13.28 (s, 1 H, NH), 12.04 (s, 1 H,

COOH), 10.86 (s, 1 H, NH), 7.76 (d, J = 8 Hz, 1 H, H-4), 7.68

(s, 1 H, H-vinyl), 7.34 (t, J = 8 Hz, 1 H), 7.28 (dd, J = 2, 8

Hz, 1 H, H-5), 7.08 (d, J = 2 Hz, 1 H, H-7), 7.18, 7.13, 6.9
(m, 4 H, Ar-H), 4.1 (q, J = 7 Hz, 2 H, OC$\underline{H}_2$CH$_3$), 2.96 (t, J =
7.5 Hz, 2 H, CH$_2$), 2.43 (t, J = 7.5 Hz, 2 H, CH$_2$), 2.05 (s, 3
H, CH$_3$), 1.35 (t, J = 7 Hz, 3 H, OCH$_2$C$\underline{H}_3$).

5   **5.   BIOLOGICAL EVALUATION**

It will be appreciated that, in any given series of
compounds, a spectrum of biological activity will be afforded.
In its presently preferred embodiments, this invention relates to
3-methylidenyl-2-indolinones which demonstrate the ability to
10   modulate RTK, CTK, and STK activity. The assays employed to
select those compounds demonstrating of the desired activity are
set forth below. Table 2 shows the results of testing of
compounds of this invention using the described assays. The
results shown are not to be construed as limiting the scope of
15   this invention in any manner whatsoever.

TABLE 2

Compound	HER-2 kinase IC50 (µM)	BrdU HER 2 driven IC50 (µM)	BrdU EGF driven IC50 (µM)	SKOV3 growth (2%FBS) IC50 (µM)	A431 growth (2%FBS) IC50 (µM)	bio FLK1 IC50 (µM) % if inhibition at 100 (µM)	bio flkGST IC50 (µM)	bio fgfR1 IC50 (µM)	bio PDGF IC50 (µM)	bio EGFr IC50 (µM)	bio pyk2 IC50 (µM)	cell EGF IC50 (µM)	bio src IC50 (µM)	HUVEC-VEGF IC50 (µM)	HUVEC aFGF IC50 (µM)	PDGF-Induced BrdU incorp. IC50 (µM)	FGF-Induced BrdU incorp. IC50 (µM)
1	13.8	8.7	6.8	5.5	5.4	>50			>100	>100		45.7					
2	13.7	ND	8.5	4.8	4.5	>50			>100	>100		>100					
3	6.4	9.1	11	2.6	2.2												
4	5.8	4.4	26.6	7	5.1					>100		20.06					
5	6.5	10.5	7.2	5.4	4.3					>100		21.47					
6	15.3	4.7	12	19.4	2.9					>100		40.47					
7	>100	16.5	24.5	2.5	1.2					>100		40.37					
8	8.36	33.4	>50	0.96	0.95					>100		43.68					
9	>100	4.5	11	14.5	17.3					>100		34.59					
10	45.78	14.1	29	11.2	8.5					>100		58.07					
11	57.3	9.3	23.3	15	12.5		24.12		>100	>100		72.94					
12	>100	31.9	30.6	10.1	7.9					>100		71.92					
13	>100	9	28.8	21.3	9				>100	>100		30.78					
14	22.7	ND	ND	ND	ND					>100		>100					
15	19	4.4	7.8	7	4.8				26.52	>100		48.52					
16	9.9	4.2	6.4	5.9	4.1				>100	>100		44.46					
17	7.8	5.5	7.4	6.1	4.3					>100		67.98					
18	8.4	>50	>50	1	0.6				86.93	>100		>100					
19	9.2	3.3	9.3	5.9	4.3					>100		34.80					
20	38.6	15.7	30	35.5	29.5					>100		78.08					
21	11.8	6.6	8	13	15.3					>100		18.45					
22	12.4	ND	ND	ND	ND					>100		>100					
23	33.2	27.1	32.9	17.8	14.5					>100		37.71					
24	13.6	7.1	7.5	6.5	4.5				>100	>100		38.47					
26	15.3	>50	>50	5.4	3					>100		93.99					
27	15.8	23.4	26.8	5.1	6.4				>100	>100		30.58					
28	16.92									>100		>100					

Compound	HER-2 kinase IC$_{50}$ (µM)	BrdU HER 2 driven IC$_{50}$ (µM)	BrdU EGF driven IC$_{50}$ (µM)	SKOV3 growth (2%FBS) IC$_{50}$ (µM)	A431 growth (2%FBS) IC$_{50}$ (µM)	bio FLK1 IC$_{50}$ (µM) % if inhibition at 100 (µM)	bio flkGST IC$_{50}$ (µM)	bio fgfR1 IC$_{50}$ (µM)	bio PDGF IC$_{50}$ (µM)	bio EGFr IC$_{50}$ (µM)	bio pyk2 IC$_{50}$ (µM)	cell EGF IC$_{50}$ (µM)	bio src IC$_{50}$ (µM)	HUVEC-VEGF IC$_{50}$ (µM)	HUVEC aFGF IC$_{50}$ (µM)	PDGF-Induced BrdU Incorp. IC$_{50}$ (µM)	FGF-Induced BrdU Incorp. IC$_{50}$ (µM)
29	17.1	8.1	15.2	7.9	5.6				>100	>100		>100					
30	28.02	8.1	30.3	16	9.9				>100	>100		63.44					
31	12.92	28.7	38.6	0.4	0.3				>100	>100		29.57					
32	90.18	ND	ND	ND	ND					>100		>100					
33	20.2	ND	ND	10.3	6.7				>100	>100		53.19					
34	>100	3.5	11	7.6	4.4				56.68	>100		38.19					
35	20.7	13.8	28.1	16.2	13.4				>100	>100		>100					
36	20.7	21.4	31	>25	>25				>100	>100		66.81					
37	22.5	ND	ND	ND	ND				>100	>100		>100					
38	32.3	ND	ND	ND	ND					>100		>100					
39	33.7	5.4	16.2	9.6	9.2				>100	>100		>100					
40	36.6	22.6	28.6	22.5	14.2				>100	>100		78.95					
41	39.8	ND	ND	ND	ND				>100	>100		>100					
42	47.5	ND	ND	ND	ND				43.08	>100		>100					
43	52.9	ND	ND	ND	ND				10.99	>100		>100					
44	53.6	ND	ND	ND	ND				>100	>100		76.93					
45	56.9	>50	>50	ND	3.2					23.99		>100					
46	59.9	25.3	45.7	7.7	5.5				>100	>100		>100					
47	60.6	>50	>50	46.9	40.4					36.82		>100					
48	65	ND	ND	ND	ND					>100		>100					
49	65.5	9.1	24.1	20.6	20.7				>100	>100		>100					
50	67.6	31.1	37.9	ND	ND				>100	>100		>100					
51	71.8	ND	ND	14.5	ND					>100		>100					
52	72.8	ND	ND	ND	ND					>100		>100					
53	80.7	ND	ND	ND	ND					>100		85.04					
54	85.6	ND	ND	ND	ND					>100		79.32					
55	89.3	ND	ND	ND	ND				>100	>100		78.36					
56	96.2	ND	ND	>20	ND					10.1		>100					
57	>100	9.6	12.3	4	5.8				>100	>100		>100					
58	>100	13.5	27.5	22	10.5				>100	>100		>100					
59	>100	14.4	44.5	17.2	6				>100	>100		>100					

Compound	HER-2 kinase IC50 (µM)	BrdU HER 2 driven IC50 (µM)	BrdU EGF driven IC50 (µM)	SKOV3 growth (2%FBS) IC50 (µM)	A431 growth (2%FBS) IC50 (µM)	bio FLK1 IC50 (µM) % if inhibition at 100 (µM)	bio flkGST IC50 (µM)	bio fgfR1 IC50 (µM)	bio PDGF IC50 (µM)	bio EGFr IC50 (µM)	bio pyk2 IC50 (µM)	cell EGF IC50 (µM)	bio src IC50 (µM)	HUVEC-VEGF IC50 (µM)	HUVEC aFGF IC50 (µM)	PDGF-Induced BrdU Incorp. IC50 (µM)	FGF-Induced BrdU Incorp. IC50 (µM)
60	>100	40	>50	4.8	5.9				24.49	>100		>100					
61	>100	>50	>50	>25	>25				>100	>100		>100					
62	>100	28.8	>50	6.1	8				>100	>100		>100					
63	>100	20.4	30.5	>25	11				>100	>100		>100					
64	>100								>100	>100		>100					
65	>100									>100		>100					
66	>100									>100		>100					
67	>100									>100		>100					
68	>100									>100		>100					
69	>100									>100		>100					
70	>100									22.6		>100					
71	>100									29.6		>100					
72	>100									11.8		>100					
73	>50									7.9		>50					
74	>100						2.15			13.2		>100					
107	>100						1.08	6.16	<0.78	>100		>100					
108	>100						2.68	2.57	2.97	>100		>100					
109	>100						3.05	>20	2.79	>100		>100					
110	>100						0.43	17.48	3.15	>100		>100					
111	>100						2.36	0.47	0.01	>100		>100					
112							3.31	>20	0.02	>100		>100					
114	61.73						0.1	4.26	0.05	>100		>100					
116	6.07						13.54	0.29	0.02	>100		>100					
117							9.29	>20	>100		4.24						
118							6.33		>100		2.02						
119							6.32		>100								
120							1		9.97								
121							0.51		26.66								
122							16.88		2.13								
123							13.91		15.21		4.71						
124									33.89		4.21						

Compound	HER-2 kinase IC50 (µM)	BrdU HER 2 driven IC50 (µM)	BrdU EGF driven IC50 (µM)	SKOV3 growth (2%FBS) IC50 (µM)	A431 growth (2%FBS) IC50 (µM)	bio FLK1 IC50 (µM) % if inhibition at 100 (µM)	bio flkGST IC50 (µM)	bio fgfR1 IC50 (µM)	bio PDGF IC50 (µM)	bio EGFr IC50 (µM)	bio pyk2 IC50 (µM)	cell EGF IC50 (µM)	bio src IC50 (µM)	HUVEC-VEGF IC50 (µM)	HUVEC aFGF IC50 (µM)	PDGF-Induced BrdU incorp. IC50 (µM)	FGF-Induced BrdU incorp. IC50 (µM)
125									5.73								
126							14.8		63.28								
127									0.96								
128									17.11								
129									31.69								
130							12.88		2.32								
131									23.18		2.18						
132									0.65		0.22						
133							8.18		2.69								
134									4.88								
135							0.56		0.5	0.19							
136							7.54		26.95		2.77						
137							17.65		6.17	>100	>10						
138							7.58		28.24	>100	2.18						
139							6.65	12.57	>100	>100							
140							11.53	12.83	>100	>100							
141							21.14	8.38	85.11	>100							
142							26.92	5.9	>100	>100							
143							9.03	16.56	>100	>100							
144							11.79	2.77	>100	>100							
145							15.6	15.57	>100	>100							
146							15.26	>20	>100	>100							
147							23.94	>20	>100	>100							
148								>20	>100	>100							
149							2.66	0.77	5.26		5.02						
150							0.2		0.34		0.01						
151							0.71		0.65								
152	>100							>20	>100	>100							
153	>100							>20	>100	>100							
154	>100							>20	>100	>100			>100				
155	>100								>100	>100							
156	>100						4.2		8.32	8.2	0.22		0.15				
157	>100							>20	>100	30.8			>100				
158							0.26		18.03	>100	12.35	>100					
159							2.87		74.08	>100	9.6	>100					
160						1.53	0.12	5.27	2.72	>100	0.68	>100	9				

Compound	HER-2 kinase IC50 (µM)	BrdU HER 2 driven IC50 (µM)	BrdU EGF driven IC50 (µM)	SKOV3 growth (2%FBS) IC50 (µM)	A431 growth (2%FBS) IC50 (µM)	bio FLK1 IC50 (µM) % if inhibition at 100 (µM)	bio flkGST IC50 (µM)	bio fgfR1 IC50 (µM)	bio PDGF IC50 (µM)	bio EGFr IC50 (µM)	bio pyk2 IC50 (µM)	cell EGF IC50 (µM)	bio src IC50 (µM)	HUVEC-VEGF IC50 (µM)	HUVEC aFGF IC50 (µM)	PDGF-Induced BrdU Incorp. IC50 (µM)	FGF-Induced BrdU Incorp. IC50 (µM)
161						2.1	<0.008	>20	25.06	>100	3.4	>100					
162								>20	8.02	>100		>100					
163								>20	>100	>100		>100					
164	>100							>20	>100	>100		>100					
165	>100							>20	48.71	>100		>100					
166	>100							>20	>100	>100		>100					
167								>20	>100	>100		>100					
168								>20	>100	17.8							
169						15.8		>20	>100	>100							
170						10.7		>20	>100	>100							
171						20.35		>20	68.56	>100		>100					
172							10.47	0.28	89.41	>100	14.75	>100					
173							11.05	0.29	69.14	22.04	9.8	>100					
174							1.85	0.41	2.5	>100	2.57	>100					
175							1.51	0.73	55.38	>100	5.53	>100					
176							2.08	0.75	79.22	>100	66.08	>100					
177							1.34	1.6	>100	6.13	>100	>100					
178							>20	3.23	>100	>100	57.09	>100					
179							1.84	4.52	>100	>100	>100	>100					
180							1.11	5.42	1.11	>100	<0.78	>100					
181							12.53	5.74	>100	>100	>100	>100					
182							18.68	5.94	5.4	>100	37.09	>100					
183							1.55	7.68	>100	>100	>100	>100					
184							>20	8.6	>100	>100	>100	>100					
185							3.39	9.43	>100	>100	>100	>100					
186							6.12	10.38	>100	>100	>100	>100					
187							>20	13.82	>100	>100	75.8	>100					
188							>20	17.47	>100	>100	86.41	>100					
189							>20	>20	>100	>100	>100	>100					
190						0.06	0.087	5.85	2.08	>100	<0.78		0.11	0.0019	0.0159	2.9	18.2
191						0.02	0.04	0.04	0.05	>100	68.5	>100	11.96	0.00004	0.25	1.1	23.6
192						0.03	0.03	1.81	0.004	>100	4.87	>100	10.49	0.0003	0.076	1.4	15.7

Compound	HER-2 kinase IC50 (µM)	BrdU HER 2 driven IC50 (µM)	BrdU EGF driven IC50 (µM)	SKOV3 growth (2%FBS) IC50 (µM)	A431 growth (2%FBS) IC50 (µM)	bio FLK1 IC50 (µM) % if inhibition at 100 (µM)	bio flkGST IC50 (µM)	bio fgfR1 IC50 (µM)	bio PDGF IC50 (µM)	bio EGFr IC50 (µM)	bio pyk2 IC50 (µM)	cell EGF IC50 (µM)	bio src IC50 (µM)	HUVEC-VEGF IC50 (µM)	HUVEC aFGF IC50 (µM)	PDGF-Induced BrdU Incorp. IC50 (µM)	FGF-Induced BrdU Incorp. IC50 (µM)
193						0.02	3.60	0.02	44.55	90.72	17.32		48.59	0.00094	0.0025	13.5	26.7
194						0.001	6.67	0.04	81.35		4.09		20.58	0.001	0.0044	14.2	30.1
195						0.03	4.35	0.02	2.04	36.48	10.70	>100	0.26		1.6	3.7	30.2
196						0.89	0.41	0.05	33.68	>100	2.37		11.25	0.02	202	3	42.1
197						2.10	0.61		>100	>100	11.98	>100		<0.003	1.7	4.9	>50
198						7.44	5.84		0.04	>100	1.5	>100	2.34	0.29	0.1	4.2	>50
199						0.13	0.17		0.89	>100	2.83	>100		<0.03	9.2	3.5	>50
200						0.97	1.29		11.80	>100	5.62	>100		0.22	0.16	8.6	>50
201						0.16	0.57		1.73	>100	6.61	>100		<0.03	3.9		>50
202						0.63	2.54	2.27	9.58	>100		>100	>50	<0.03	2.1	5.2	>50
203						0.14			0.15	>12.5		>12.5					
204						0.36	0.07	0.59	0.68	7.09		>100	4.44				
205						0.52	0.44	2.02	5.43	16.21		>100	1.34				
206						6.08	0.06	1.18	0.04	>100		>100	0.74				
207						2.72	1.50	7.70	0.87	>100		>100	7.52				
208						<0.78	0.38		23.45	>100	0.13	>100	1.84				
209								30.24	84.59	>100							
214						50.84			>100	>100		>100	>100				
215			>100			0.02	0.08	0.07	0.50	>100	6.90	>100	5.59	0.07	1.1	20	25
216			>50			2.91			0.22	>100	23.86	>100	4.75	<0.07	1.4	N/A*	N/A*
217			>50			1.47			0.21	>100	2.89	>100	3.68	0.62	4.8	8.7	>50
218			43.5			0.20	0.48	0.10	2.11	98.06	32.27	>100		<0.07	0.35	24.8	>50
219			>50			0.19	0.11		0.47	>100	4.15	>100	0.08	0.016	0.26	7.9	>50
220			>50			0.35	0.18	0.09	0.62	>100	1.89	>100	8.48	0.04	0.49	8.5	>50
222						<0.78	0.57		3.75	13.29		>100	5.77			>50	35
223						28.25		1.22	0.781				1.06				
224						1.0	0.67	4.24	2.38	>100		>100	1.16				

A.   Assay Procedures.

The following in vitro assays may be used to determine the level of activity and effect of the different compounds of the present invention on one or more of the PKs. Similar assays can be designed along the same lines for any PK using techniques well known in the art.

The cellular/catalytic assays described herein are performed in an ELISA format. The general procedure is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for a selected period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor is added. The cells are lysed and the lysate is transferred to the wells of an ELISA plate previously coated with a specific antibody recognizing the substrate of the enzymatic phosphorylation reaction. Non-substrate components of the cell lysate are washed away and the amount of phosphorylation on the substrate is detected with an antibody specifically recognizing phosphotyrosine compared with control cells that were not contacted with a test compound. The cellular/biologic assays described herein measure the amount of DNA made in response to activation of a test kinase, which is a general measure of a proliferative response. The general procedure for this assay is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for a selected period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor is added. After incubation at least overnight, a DNA labeling reagent such as Bromodeoxyuridine (BrdU) or 3H-thymidine is added. The amount of labeled DNA is detected with either an anti-BrdU antibody or by measuring radioactivity and is compared to control cells not contacted with a test compound.

Cellular/Catalytic Assays

Enzyme linked immunosorbent assays (ELISA) may be used to detect and measure the presence of PK activity. The ELISA may be conducted according to known protocols which are described

5   in, for example, Voller, et al., 1980, "Enzyme-Linked Immunosorbent Assay," In: Manual of Clinical Immunology, 2d ed., edited by Rose and Friedman, pp 359-371 Am. Soc. Of Microbiology, Washington, D.C.

The disclosed protocol may be adapted for determining

10 activity with respect to a specific PK. That is, the preferred protocols for conducting the ELISA experiments for specific PKs is provided below. However, adaptation of these protocols for determining a compound's activity for other members of the RTK family, as well as for CTKs and STKs, is

15 well within the scope of knowledge of those skilled in the art.

FLK-1 Assay

An ELISA assay is conducted to measure the kinase activity of the FLK-1 receptor and more specifically, the

20 inhibition or activation of TK activity on the FLK-1 receptor. Specifically, the following assay can be conducted to measure kinase activity of the FLK-1 receptor in cells genetically engineered to express Flk-1.

Materials and Reagents.

25   a.   Corning 96-well ELISA plates (Corning Catalog No. 25805-96),

        b.   Cappel goat anti-rabbit IgG (catalog no. 55641),

        c.   PBS (Gibco Catalog No. 450-1300EB),

        d.   TBSW Buffer (50 mM Tris (pH 7.2), 150 mM NaCl and

30 0.1% Tween-20),

        e.   Ethanolamine stock (10% ethanolamine (pH 7.0), stored at 4°C),

        f.   HNTG buffer (20mM HEPES buffer (pH 7.5), 150mM NaCl, 0.2% Triton X-100, and 10% glycerol),

g.   EDTA (0.5 M (pH 7.0) as a 100X stock),

h.   Sodium orthovanadate (0.5 M as a 100X stock),

i.   Sodium pyrophosphate (0.2 M as a 100X stock),

j.   NUNC 96 well V bottom polypropylene plates (Applied
Scientific Catalog No. AS-72092),

k.   NIH3T3 C7#3 Cells (FLK-1 expressing cells),

l.   DMEM with 1X high glucose L-Glutamine (catalog No.
11965-050),

m.   FBS, Gibco (catalog no. 16000-028),

n.   L-glutamine, Gibco (catalog no. 25030-016),

o.   VEGF, PeproTech, Inc. (catalog no. 100-20)(kept as 1
μg/100 μl stock in Milli-Q dH$_2$O and stored at -20° C,

p.   Affinity purified anti-FLK-1 antiserum,

q.   UB40 monoclonal antibody specific for
phosphotyrosine (see, Fendley, et al., 1990, Cancer Research
50:1550-1558),

r.   EIA grade Goat anti-mouse IgG-POD (BioRad catalog
no. 172-1011),

s.   2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid
(ABTS) solution (100mM citric acid (anhydrous), 250 mM Na$_2$HPO$_4$
(pH 4.0), 0.5 mg/ml ABTS (Sigma catalog no. A-1888)), solution
should be stored in dark at 4° C until ready for use,

t.   H$_2$O$_2$ (30% solution)(Fisher catalog no. H325),

u.   ABTS/ H$_2$O$_2$ (15ml ABTS solution, 2 μl H$_2$O$_2$) prepared 5
minutes before use and left at room temperature,

v.   0.2 M HCl stock in H$_2$O,

w.   dimethylsulfoxide (100%)(Sigma Catalog No. D-8418),
and

y.   Trypsin-EDTA (Gibco BRL Catalog No. 25200-049).

Protocol.

1.   Coat Corning 96-well ELISA plates with 1.0 μg per
well Cappel Anti-rabbit IgG antibody in 0.1M Na$_2$CO$_3$, pH 9.6.
Bring final volume to 150 μl per well. Coat plates overnight

at 4°C. Plates can be kept up to two weeks when stored at
4°C.

2.   Grow cells in Growth media (DMEM, supplemented with
2.0 mM L-Glutamine, 10% FBS) in suitable culture dishes until
5   confluent at 37°C, 5% CO_2.

3.   Harvest cells by trypsinization and seed in Corning
25850 polystyrene 96-well round bottom cell plates, 25,000
cells/well in 200 µl of growth media.

4.   Grow cells at least one day at 37°C, 5% CO_2.

10   5.   Wash cells with D-PBS 1X.

6.   Add 200 µl/well of starvation media (DMEM, 2.0mM 1-
Glutamine, 0.1% FBS). Incubate overnight at 37°C, 5% CO_2.

7.   Dilute Compounds 1:20 in polypropylene 96 well
plates using starvation media. Dilute dimethylsulfoxide 1:20
15   for use in control wells.

8.   Remove starvation media from 96 well cell culture
plates and add 162 µl of fresh starvation media to each well.

9.   Add 18 µl of 1:20 diluted compound dilution (from
step 7) to each well plus the 1:20 dimethylsulfoxide dilution
20   to the control wells (+/- VEGF), for a final dilution of 1:200
after cell stimulation. Final dimethylsulfoxide is 0.5%.
Incubate the plate at 37°C, 5% CO_2 for two hours.

10.   Remove unbound antibody from ELISA plates by
inverting plate to remove liquid. Wash 3 times with TBSW +
25   0.5% ethanolamine, pH 7.0. Pat the plate on a paper towel to
remove excess liquid and bubbles.

11.   Block plates with TBSW + 0.5% ethanolamine, pH 7.0,
150 µl per well. Incubate plate thirty minutes while shaking
on a microtiter plate shaker.

30   12.   Wash plate 3 times as described in step 10.

13.   Add 0.5 µg/well affinity purified anti-FLU-1
polyclonal rabbit antiserum. Bring final volume to 150

µl/well with TBSW + 0.5% ethanolamine pH 7.0. Incubate plate for thirty minutes while shaking.

14. Add 180 µl starvation medium to the cells and stimulate cells with 20 µl/well 10.0 mM sodium orthovanadate and 500 ng/ml VEGF (resulting in a final concentration of 1.0 mM sodium orthovanadate and 50 ng/ml VEGF per well) for eight minutes at 37°C, 5% CO_2. Negative control wells receive only starvation medium.

15. After eight minutes, media should be removed from the cells and washed one time with 200 µl/well PBS.

16. Lyse cells in 150 µl/well HNTG while shaking at room temperature for five minutes. HNTG formulation includes sodium ortho vanadate, sodium pyrophosphate and EDTA.

17. Wash ELISA plate three times as described in step 10.

18. Transfer cell lysates from the cell plate to ELISA plate and incubate while shaking for two hours. To transfer cell lysate pipette up and down while scrapping the wells.

19. Wash plate three times as described in step 10.

20. Incubate ELISA plate with 0.02 µg/well UB40 in TBSW + 05% ethanolamine. Bring final volume to 150 µl/well. Incubate while shaking for 30 minutes.

21. Wash plate three times as described in step 10.

22. Incubate ELISA plate with 1:10,000 diluted EIA grade goat anti-mouse IgG conjugated horseradish peroxidase in TBSW plus 0.5% ethanolamine, pH 7.0. Bring final volume to 150 µl/well. Incubate while shaking for thirty minutes.

23. Wash plate as described in step 10.

24. Add 100 µl of ABTS/H_2O_2 solution to well. Incubate ten minutes while shaking.

25. Add 100 µl of 0.2 M HCl for 0.1 M HCl final concentration to stop the color development reaction. Shake 1

minute at room temperature. Remove bubbles with slow stream of air and read the ELISA plate in an ELISA plate reader at 410 nm.

EGF Receptor-HER2 Chimeric Receptor Assay In Whole Cells.

HER2 kinase activity in whole EGFR-NIH3T3 cells are measured as described below:

Materials and Reagents.

a.   EGF: stock concentration: 16.5 ILM, EGF 201, TOYOBO, Co., Ltd. Japan.

b.   05-101 (UBI) (a monoclonal antibody recognizing an EGFR extracellular domain).

c.   Anti-phosphotyrosine antibody (anti-Ptyr) (polyclonal)(see, Fendley, et al., supra).

d.   Detection antibody: Goat anti-rabbit lgG horseradish peroxidase conjugate, TAGO, Inc., Burlingame, CA.

e.   TBST buffer:

Tris-HCl, pH 7.2	50 mM
NaCl	150 mM
Triton X-100	0.1

f.   HNTG 5X stock:

HEPES	0.1 M
NaCl	0.75 M
Glycerol	50%
Triton X-100	1.0%

g.   ABTS stock:

Citric Acid	100 mM
Na_2HPO_4	250 mM
HCl, conc.	0.5 mM
ABTS*	0.5mg/ml

*   (2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid)). Keep solution in dark at 4°C until use.

h.   Stock reagents of:

EDTA 100 mM pH 7.0

Na$_3$VO$_4$ 0.5 M

Na$_4$(P$_2$O$_7$) 0.2 M

Procedure.

5   **Pre-coat ELISA Plate**

       1.   Coat ELISA plates (Corning, 96 well, Cat. #25805-96) with 05-101 antibody at 0.5 µg per well in PBS, 100 µl final volume/well, and store overnight at 4°C. Coated plates are good for up to 10 days when stored at 4°C.

10   2.   On day of use, remove coating buffer and replace with 100 µl blocking buffer (5% Carnation Instant Non-Fat Dry Milk in PBS). Incubate the plate, shaking, at room temperature (about 23°C to 25°C) for 30 minutes. Just prior to use, remove blocking buffer and wash plate 4 times with TBST

15 buffer.

       Seeding Cells

       1.   An NIH3T3 cell line overexpressing a chimeric receptor containing the EGFR extracellular domain and intracellular HER2 kinase domain can be used for this assay.

20   2.   Choose dishes having 80-90% confluence for the experiment. Trypsinize cells and stop reaction by adding 10% fetal bovine serum. Suspend cells in DMEM medium (10% CS DMEM medium) and centrifuge once at 1500 rpm, at room temperature for 5 minutes.

25   3.   Resuspend cells in seeding medium (DMEM, 0.5% bovine serum), and count the cells using trypan blue. Viability above 90% is acceptable. Seed cells in DMEM medium (0.5% bovine serum) at a density of 10,000 cells per well, 100 µl per well, in a 96 well microtiter plate. Incubate seeded

30 cells in 5% CO$_2$ at 37°C for about 40 hours.

       Assay Procedures

       1.   Check seeded cells for contamination using an inverted microscope. Dilute drug stock (10 mg/ml in DMSO) 1:10

in DMEM medium, then transfer 5 µl to a TBST well for a final
drug dilution of 1:200 and a final DMSO concentration of 1%.
Control wells receive DMSO alone.   Incubate in 5% CO_2 at 37°C
for two hours.

5   2.   Prepare EGF ligand: dilute stock EGF in DMEM so
that upon transfer of 10 µl dilute EGF (1:12 dilution), 100 nM
final concentration is attained.

        3.   Prepare fresh HNTG* sufficient for 100 µl per
well, and place on ice.

10   HNTG* (10 ml):

        HNTG stock   2.0 ml
        milli-Q H_2O 7.3 ml
        EDTA, 100 mM, pH 7.0   0.5 ml
        Na_3VO_4 (0.5 M)   0.1 ml
15      $Na_4(P_2O_7)$ (0.2 M)   0.1 ml

        4.   After 120 minutes incubation with drug, add
prepared SGF ligand to cells, 10 µl per well, to a final
concentration of 100 nM. Control wells receive DMEM alone.
Incubate with shaking, at room temperature, for 5 minutes.

20   5.   Remove drug, EGF, and DMEM. Wash cells twice
with PBS. Transfer HNTG* to cells, 100 µl per well. Place on
ice for 5 minutes.   Meanwhile, remove blocking buffer from
other ELISA plate and wash with TBST as described above.

        6.   With a pipette tip securely fitted to a
25 micropipettor, scrape cells from plate and homogenize cell
material by repeatedly aspirating and dispensing the HNTG*
lysis buffer. Transfer lysate to a coated, blocked, and
washed ELISA plate. Incubate shaking at room temperature for
one hour.

30   7.   Remove lysate and wash 4 times with TBST.
Transfer freshly diluted anti-Ptyr antibody to ELISA plate at
100 µl per well. Incubate shaking at room temperature for 30
minutes in the presence of the anti-Ptyr antiserum (1:3000

dilution in TBST).

8.   Remove the anti-Ptyr antibody and wash 4 times
with TBST. Transfer the freshly diluted TAGO anti-rabbit IgG
antibody to the ELISA plate at 100 μl per well. Incubate
shaking at room temperature for 30 minutes (anti-rabbit IgG
antibody: 1:3000 dilution in TBST).

9.   Remove TAGO detection antibody and wash 4 times
with TBST. Transfer freshly prepared ABTS/H_2O_2 solution to
ELISA plate, 100 μl per well. Incubate shaking at room
temperature for 20 minutes. (ABTS/H_2O_2 solution: 1.0 μl 30% H_2O_2
in 10 ml ABTS stock).

10.   Stop reaction by adding 50 μl 5N H_2SO_4
(optional), and determine O.D. at 410 nm.

11.   The maximal phosphotyrosine signal is
determined by subtracting the value of the negative controls
from the positive controls. The percent inhibition of
phosphotyrosine content for extract-containing wells is then
calculated, after subtraction of the negative controls.

PDGF-R Assay

All cell culture media, glutamine, and fetal bovine
serum can be purchased from Gibco Life Technologies (Grand
Island, NY) unless otherwise specified. All cells are grown
in a humid atmosphere of 90-95% air and 5-10% CO_2 at 37°C.
All cell lines are routinely subcultured twice a week and
are negative for mycoplasma as determined by the Mycotect
method (Gibco).

For ELISA assays, cells (U1242, obtained from Joseph
Schlessinger, NYU) are grown to 80-90% confluency in growth
medium (MEM with 10% FBS, NEAA, 1 mM NaPyr and 2 mM GLN) and
seeded in 96-well tissue culture plates in 0.5% serum at
25,000 to 30,000 cells per well. After overnight incubation
in 0.5% serum-containing medium, cells are changed to serum-
free medium and treated with test compound for 2 hr in a 5%

CO_2, 37°C incubator.   Cells are then stimulated with ligand
for 5-10 minute followed by lysis with HNTG (20 mM Hepes,
150 mM NaCl, 10% glycerol, 5 mM EDTA, 5 mM Na_3VO_4, 0.2%
Triton X-100, and 2 mM NaPyr).   Cell lysates (0.5 mg/well in

5   PBS) are transferred to ELISA plates previously coated with
receptor-specific antibody and which had been blocked with
5% milk in TBST (50 mM Tris-HCl pH 7.2, 150 mM NaCl and 0.1%
Triton X-100) at room temperature for 30 min.   Lysates are
incubated with shaking for 1 hour at room temperature.   The

10   plates are washed with TBST four times and then incubated
with polyclonal anti-phosphotyrosine antibody at room
temperature for 30 minutes.   Excess anti-phosphotyrosine
antibody is removed by rinsing the plate with TBST four
times.   Goat anti-rabbit IgG antibody is added to the ELISA

15   plate for 30 min at room temperature followed by rinsing
with TBST four more times.   ABTS (100 mM citric acid, 250 mM
Na_2HPO_4 and 0.5 mg/mL 2,2'-azino-bis(3-ethylbenzthiazoline-6-
sulfonic acid)) plus H_2O_2 (1.2 mL 30% H_2O_2 to 10 ml ABTS) is
added to the ELISA plates to start color development.

20   Absorbance at 410 nm with a reference wavelength of 630 nm
is recorded about 15 to 30 min after ABTS addition.

IGF-1 RECEPTOR Assay

The following protocol may be used to measure
phosphotyrosine level on IGF-1 receptor, which indicates IGF-1

25   receptor tyrosine kinase activity.

Materials and Reagents.

a.   The cell line used in this assay is 3T3/IGF-1R, a
cell line genetically engineered to overexpresses IGF-1
receptor.

30   b.   NIH3T3/IGF-1R is grown in an incubator with 5% CO_2 at
37°C. The growth media is DMEM + 10% FBS (heat inactivated)+
2mM L-glutamine.

c.   Affinity purified anti-IGF-1R antibody 17-69.

d.   D-PBS:

KH_2PO_4	0.20 g/l	
KH_2PO_4	2.16 g/l	
KCl	0.20 g/l	
NaCl	8.00 g/l (pH 7.2)	

e. Blocking Buffer: TBST plus 5% Milk (Carnation
Instant Non-Fat Dry Milk).

f. TBST buffer:

Tris-HCl	50 mM
NaCl	150mM (pH 7.2/HCl 10N)
Triton X-100	0.1%

Stock solution of TBS (10X) is prepared, and Triton
X-100 is added to the buffer during dilution.

g. HNTG buffer:

HEPES	20 mM
NaCl	150 mM (pH 7.2/HCl 1N)
Glycerol	10%
Triton X-100	0.2%

Stock solution (5X) is prepared and kept at 4°C.

h. EDTA/HCl: 0.5 M pH 7.0 (NaOH) as 100X stock.

i. Na_3VO_4: 0.5 M as 100X stock and aliquots are kept at
80°C.

j. $Na_4P_2O_7$: 0.2 M as 100X stock.

k. Insulin-like growth factor-1 from Promega (Cat#
G5111).

l. Rabbit polyclonal anti-phosphotyrosine antiserum.

m. Goat anti-rabbit IgG, POD conjugate (detection
antibody), Tago (Cat. No. 4520, Lot No. 1802): Tago, Inc.,
Burlingame, CA.

n. ABTS (2,2'-azinobis(3-ethylbenzthiazolinesulfonic
acid)) solution:

Citric acid	100 mM
Na_2HPO_4	250 mM (pH 4.0/1 N HCl)
ABTS	0.5 mg/ml

ABTS solution should be kept in dark and 4°C. The

solution should be discarded when it turns green.

o.   Hydrogen Peroxide: 30% solution is kept in the dark and at 4°C.

Procedure.

All the following steps are conducted at room temperature unless specifically indicated otherwise. All ELISA plate washings are performed by rinsing the plate with tap water three times, followed by one TBST rinse. Pat plate dry with paper towels.

Cell Seeding:

1.   The cells, grown in tissue culture dish (Corning 25020-100) to 80-90% confluence, are harvested with Trypsin-EDTA (0.25%, 0.5 ml/D-100, GIBCO).

2.   Resuspend the cells in fresh DMEM + 10% FBS + 2mM L-Glutamine, and transfer to 96-well tissue culture plate (Corning, 25806-96) at 20,000 cells/well (100 µl/well). Incubate for 1 day then replace medium to serum-free medium (90/µl) and incubate in 5% CO_2 and 37°C overnight.

ELISA Plate Coating and Blocking:

1.   Coat the ELISA plate (Corning 25805-96) with Anti-IGF-1R Antibody at 0.5 µg/well in 100 µl PBS at least 2 hours.

2.   Remove the coating solution, and replace with 100 µl Blocking Buffer, and shake for 30 minutes. Remove the blocking buffer and wash the plate just before adding lysate.

Assay Procedures:

1.   The drugs are tested under serum-free condition.

2.   Dilute drug stock (in 100% DMSO) 1:10 with DMEM in 96-well poly-propylene plate, and transfer 10 µl/well of this solution to the cells to achieve final drug dilution 1:100, and final DMSO concentration of 1.0%. Incubate the cells in 5% CO_2 at 37°C for 2 hours.

3.   Prepare fresh cell lysis buffer (HNTG*)

HNTG	2 ml
EDTA	0.1 ml
Na_3VO_4	0.1 ml
$Na_4(P_2O_7)$	0.1 ml
H_2O	7.3 ml

5

4. After drug incubation for two hours, transfer 10 µl/well of 200nM IGF-1 Ligand in PBS to the cells (Final Conc. is 20 nM), and incubate at 5% CO_2 at 37°C for 10 minutes.

5. Remove media and add 100 µl/well HNTG* and shake for
10 10 minutes. Look at cells under microscope to see if they are adequately lysed.

6. Use a 12-channel pipette to scrape the cells from the plate, and homogenize the lysate by repeated aspiration and dispensing. Transfer all the lysate to the antibody
15 coated ELISA plate, and shake for 1 hour.

7. Remove the lysate, wash the plate, transfer anti-pTyr (1:3,000 with TBST) 100 µl/well, and shake for 30 minutes.

8. Remove anti-pTyr, wash the plate, transfer TAGO
20 (1:3,000 with TBST) 100 µl/well, and shake for 30 minutes.

9. Remove detection antibody, wash the plate, and transfer fresh ABTS/H_2O_2 (1.2 µl H_2O_2 to 10 ml ABTS) 100 µl/well to the plate to start color development.

Measure OD at 410 nm with a reference wavelength of 630
25 nm in Dynatec MR5000.

EGFR Assay

EGF Receptor kinase activity in cells genetically engineered to express human EGF-R can be measured as described below:

30 **Materials and Reagents.**

a. EGF Ligand: stock concentration = 16.5 µM, EGF 201, TOYOBO, Co., Ltd. Japan.

b. 05-101 (UBI) (a monoclonal antibody recognizing an

EGFR extracellular domain).

    c.   Anti-phosphotyosine antibody (anti-Ptyr) (polyclonal).

    d.   Detection antibody: Goat anti-rabbit lgG horse radish peroxidase conjugate, TAGO, Inc., Burlingame, CA.

    e.   TBST buffer:

Tris-HCl, pH 7	50 mM
NaCl	150 mM
Triton X-100	0.1

    f.   HNTG 5X stock:

HEPES	0.1 M
NaCl	0.75 M
Glycerol	50
Triton X-100	1.0%

    g.   ABTS stock:

Citric Acid	100 mM
Na_3VO_4	250 mM
HCl, conc.	4.0 pH
ABTS*	0.5 mg/ml

Keep solution in dark at 4°C until used.

    h.   Stock reagents of:

        EDTA 100 mM pH 7.0

        Na_3VO_4 0.5 M

        $Na_4(P_2O_7)$ 0.2 M

Procedure.

Pre-coat ELISA Plate

1.   Coat ELISA plates (Corning, 96 well, Cat. #25805-96) with 05-101 antibody at 0.5 µg per well in PBS, 150 µl final volume/well, and store overnight at 4°C. Coated plates are good for up to 10 days when stored at 4°C.

2.   On day of use, remove coating buffer and replace with blocking buffer (5% Carnation Instant NonFat Dry Milk in PBS). Incubate the plate, shaking, at room temperature (about

23°C to 25°C) for 30 minutes. Just prior to use, remove
blocking buffer and wash plate 4 times with TBST buffer.

Seeding Cells

1.   NIH 3T3/C7 cell line (Honegger, et al., Cell 51:199-
5   209, 1987) can be use for this assay.

2.   Choose dishes having 80-90% confluence for the
experiment. Trypsinize cells and stop reaction by adding 10%
CS DMEM medium. Suspend cells in DMEM medium (10% CS DMEM
medium) and centrifuge once at 1000 rpm at room temperature
10   for 5 minutes.

3.   Resuspend cells in seeding medium (DMEM, 0.5% bovine
serum), and count the cells using trypan blue. Viability above
90% is acceptable. Seed cells in DMEM medium (0.5% bovine
serum) at a density of 10,000 cells per well, 100 μl per well,
15   in a 96 well microtiter plate. Incubate seeded cells in 5% CO_2
at 37°C for about 40 hours.

Assay Procedures.

1.   Check seeded cells for contamination using an
inverted microscope. Dilute test compounds stock (10 mg/ml in
20   DMSO) 1:10 in DMEM medium, then transfer 5 μl to a test well
for a test compounds drug dilution of 1:200 and a final DMSO
concentration of 1%. Control wells receive DMSO alone.
Incubate in 5% CO_2 at 37°C for one hour.

2.   Prepare EGF ligand: dilute stock EGF in DMEM so that
25   upon transfer of 10 μl dilute EGF (1:12 dilution), 25 nM final
concentration is attained.

3.   Prepare fresh 10 ml HNTG* sufficient for 100 μl per
well wherein HNTG* comprises: HNTG stock (2.0 ml), milli-Q H_2O
(7.3 ml), EDTA, 100 mM, pH 7.0 (0.5 ml), Na_3VO_4 0.5 M (0.1 ml)
30   and $Na_4(P_2O_7)$, 0.2 M (0.1 ml).

4.   Place on ice.

5.   After two hours incubation with drug, add prepared
EGF ligand to cells, 10 μl per well, to yield a final

concentration of 25 nM. Control wells receive DMEM alone.
Incubate, shaking, at room temperature, for 5 minutes.

6.   Remove test compound, EGF, and DMEM. Wash cells
twice with PBS. Transfer HNTG* to cells, 100 µl per well.
5   Place on ice for 5 minutes. Meanwhile, remove blocking buffer
from other ELISA plate and wash with TBST as described above.

7.   With a pipette tip securely fitted to a
micropipettor, scrape cells from plate and homogenize cell
material by repeatedly aspirating and dispensing the HNTG*
10   lysis buffer. Transfer lysate to a coated, blocked, and
washed ELISA plate. Incubate shaking at room temperature for
one hour.

8.   Remove lysate and wash 4 times with TBST. Transfer
freshly diluted anti-Ptyr antibody to ELISA plate at 100 µl
15   per well. Incubate shaking at room temperature for 30 minutes
in the presence of the anti-Ptyr antiserum (1:3000 dilution in
TBST).

9.   Remove the anti-Ptyr antibody and wash 4 times with
TBST. Transfer the freshly diluted TAGO 30 anti-rabbit IgG
20   antibody to the ELISA plate at 100 µl per well. Incubate
shaking at room temperature for 30 minutes (anti-rabbit IgG
antibody: 1:3000 dilution in TBST).

10.   Remove detection antibody and wash 4 times with
TBST. Transfer freshly prepared ABTS/H_2O_2 solution to ELISA
25   plate, 100 µl per well. Incubate at room temperature for 20
minutes. ABTS/H_2O_2 solution: 1.2 µl 30% H_2O_2 in 10 ml ABTS
stock.

11.   Stop reaction by adding 50 µl 5N H_2SO_4 (optional),
and determine O.D. at 410 nm.
30   12.   The maximal phosphotyrosine signal is determined by
subtracting the value of the negative controls from the
positive controls. The percent inhibition of phosphotyrosine
content for extract-containing wells is then calculated, after

subtraction of the negative controls.

Met Autophosphorylation Assay

This assay determines Met tyrosine kinase activity by analyzing Met protein tyrosine kinase levels on the Met receptor.

Reagents

a. HNTG (5X stock solution): Dissolve 23.83 g HEPES and 43.83 g NaCl in about 350 ml dH$_2$O. Adjust pH to 7.2 with HCl or NaOH, add 500 ml glycerol and 10 ml Triton X-100, mix, add dH$_2$O to 1 L total volume. To make 1 L of 1X working solution add 200 ml 5X stock solution to 800 ml dH$_2$O, check and adjust pH as necessary, store at 4°C.

b. PBS (Dulbecco's Phosphate-Buffered Saline), Gibco Cat. # 450-1300EB (1X solution).

c. Blocking Buffer: in 500 ml dH$_2$O place 100 g BSA, 12.1 g Tris-pH7.5, 58.44 g NaCl and 10 ml Tween-20, dilute to 1 L total volume.

d. Kinase Buffer: To 500 ml dH$_2$O add 12.1 g TRIS (pH 7.2), 58.4 g NaCl, 40.7 g MgCl$_2$ and 1.9 g EGTA, bring to 1 L total volume with dH$_2$O.

e. PMSF (Phenylmethylsulfonyl fluoride), Sigma Cat. # P-7626, to 435.5 mg, add 100% ethanol to 25 ml total volume, vortex.

f. ATP (Bacterial Source), Sigma Cat. # A-7699, store powder at -20°C, to make up solution for use, dissolve 3.31 mg in 1 ml dH$_2$O.

g. RC-20H HRPO Conjugated Anti-Phosphotyrosine, Transduction Laboratories Cat. # E120H.

h. Pierce 1-Step ™ Turbo TMB-ELISA (3,3',5,5'-tetramethylbenzidine, Pierce Cat. # 34022.

i. H$_2$SO$_4$, add 1 ml conc.(18 N) to 35 ml dH$_2$O.

j. TRIS HCL, Fischer Cat. # BP152-5, to 121.14 g of material, add 600 ml MilliQ H$_2$O, adjust pH to 7.5 (or 7.2) with HCl, bring volume to 1 L with MilliQ H$_2$O.

k.   NaCl, Fischer Cat. # S271-10, make up 5M solution.

l.   Tween-20, Fischer Cat. # S337-500.

m. Na_3VO_4, Fischer Cat. # S454-50, to 1.8 g material add 80 ml MilliQ H_2O, adjust pH to 10.0 with HCl or NaOH, boil in microwave, cool, check pH, repeat procedure until pH stable at 10.0, add MilliQ H_2O to 100 ml total volume, make 1 ml aliquots and store at -80°C.

n. $MgCl_2$, Fischer Cat. # M33-500, make up 1M solution.

o.   HEPES, Fischer Cat. # BP310-500, to 200 ml MilliQ H_2O, add 59.6 g material, adjust pH to 7.5, bring volume to 250 ml total, sterile filter.

p.   Albumin, Bovine (BSA), Sigma Cat. # A-4503, to 30 grams material add sterile distilled water to make total volume of 300 ml, store at 4°C.

q.   TBST Buffer: to approx. 900 ml dH_2O in a 1 L graduated cylinder add 6.057 g TRIS and 8.766 g NaCl, when dissolved, adjust pH to 7.2 with HCl, add 1.0 ml Triton X-100 and bring to 1 L total volume with dH_2O.

r.   Goat Affinity purified antibody Rabbit IgG (whole molecule), Cappel Cat. # 55641.

s.   Anti h-Met (C-28) rabbit polyclonal IgG antibody, Santa Cruz Chemical Cat. # SC-161.

t.   Transiently Transfected EGFR/Met chimeric cells (EMR) (Komada, et al., Oncogene, 8:2381-2390 (1993).

u.   Sodium Carbonate Buffer, (Na_2CO_4, Fischer Cat. # S495): to 10.6 g material add 800 ml MilliQ H_2O, when dissolved adjust pH to 9.6 with NaOH, bring up to 1 L total volume with MilliQ H_2O, filter, store at 4°C.

Procedure

All of the following steps are conducted at room temperature unless it is specifically indicated otherwise. All ELISA plate washing is by rinsing 4X with TBST.

EMR Lysis

This procedure can be performed the night before or

immediately prior to the start of receptor capture.

1.   Quick thaw lysates in a 37° C waterbath with a
swirling motion until the last crystals disappear.

2.   Lyse cell pellet with 1X HNTG containing 1 mM PMSF.
Use 3 ml of HNTG per 15 cm dish of cells. Add 1/2 the
calculated HNTG volume, vortex the tube for 1 min., add the
remaining amount of HNTG, vortex for another min.

3.   Balance tubes, centrifuge at 10,000 x g for 10 min
at 4°C.

4.   Pool supernatants, remove an aliquot for protein
determination.

5.   Quick freeze pooled sample in dry ice/ethanol bath.
This step is performed regardless of whether lysate will be
stored overnight or used immediately following protein
determination.

6.   Perform protein determination using standard
bicinchoninic acid (BCA) method (BCA Assay Reagent Kit from
Pierce Chemical Cat. # 23225).

ELISA Procedure

1.   Coat Corning 96 well ELISA plates with 5 μg per well
Goat anti-Rabbit antibody in Carbonate Buffer for a total well
volume of 50 μl. Store overnight at 4°C.

2.   Remove unbound Goat anti-rabbit antibody by
inverting plate to remove liquid.

3.   Add 150 μl of Blocking Buffer to each well.
Incubate for 30 min. with shaking.

4.   Wash 4X with TBST. Pat plate on a paper towel to
remove excess liquid and bubbles.

5.   Add 1μg per well of Rabbit anti-Met antibody diluted
in TBST for a total well volume of 100 μl.

6.   Dilute lysate in HNTG (90 μg lysate/100μl)

7.   Add 100 μl of diluted lysate to each well. Shake
for 60 min.

8.   Wash 4X with TBST. Pat on paper towel to remove excess liquid and bubbles.

9.   Add 50 µl of 1X lysate buffer per well.

10.   Dilute compounds/extracts 1:10 in 1X Kinase Buffer in a polypropylene 96 well plate.

11.   Transfer 5.5 µl of diluted compound to ELISA plate wells. Incubate at room temperature with shaking for 20 min.

12.   Add 5.5 µl of 60 µM ATP solution per well. Negative controls do not receive any ATP. Incubate for 90 min., with shaking.

13.   Wash 4X with TBST. Pat plate on paper towel to remove excess liquid and bubbles.

14.   Add 100 µl per well of RC20 (1:3000 dilution in Blocking Buffer). Incubate 30 min. with shaking.

15.   Wash 4X with TBST. Pat plate on paper towel to remove excess liquid and bubbles.

16.   Add 100 µl per well of Turbo-TMB. Incubate with shaking for 30-60 min.

17.   Add 100 µl per well of 1M H_2SO_4 to stop reaction.

18.   Read assay on Dynatech MR7000 ELISA reader. Test Filter = 450 nm, reference filter = 410 nm.

Biochemical src assay

This assay is used to determine src protein kinase activity measuring phosphorylation of a biotinylated peptide as the readout.

Materials and Reagents:

a.   Yeast transformed with src (Sugen, Inc., Redwood City, California).

b.   Cell lysates: Yeast cells expressing src are pelleted, washed once with water, re-pelleted and stored at -80°C until use.

c.   N-terminus biotinylated EEEYEEYEEEYEEEYEEEY is prepared by standard procedures well known to those skilled in the art.

d.   DMSO: Sigma, St. Louis, MO.

e.   96 Well ELISA Plate: Corning 96 Well Easy Wash, Modified flat Bottom Plate, Corning Cat. #25805-96.

f.   NUNC 96-well V-bottom polypropylene plates for dilution of compounds: Applied Scientific Cat. # A-72092.

g.   Vecastain ELITE ABC reagent: Vector, Burlingame, CA.

h.   Anti-src (327) mab: Schizosaccharomyces Pombe is used to express recombinant Src (Superti-Furga, et al., _EMBO J._, 12:2625-2634, Superti-Furga, et al., _Nature Biochem._, 14:600-605). S. Pombe strain SP200 (h-s leul.32 ura4 ade210) is grown as described and transformations are pRSP expression plasmids are done by the lithium acetate method (Superti-Furga, _supra_). Cells are grown in the presence of 1 μM thiamine to repress expression from the nmtl promoter or in the absence of thiamine to induce expression.

i.   Monoclonal anti-phosphotyrosine, UBI 05-321 (UB40 may be used instead).

j.   Turbo TMB-ELISA peroxidase substrate: Pierce Chemical.

Buffer Solutions

a.   PBS (Dulbecco's Phosphate-Buffered Saline): GIBCO PBS, GIBCO Cat. # 450-1300EB.

b.   Blocking Buffer: 5% Non-fat milk (Carnation) in PBS.

c.   Carbonate Buffer: Na_2CO_4 from Fischer, Cat. # S495, make up 100 mM stock solution.

d.   Kinase Buffer: 1.0 ml (from 1M stock solution) $MgCl_2$, 0.2 ml (from a 1M stock solution) $MnCl_2$, 0.2 ml (from a 1M stock solution) DTT, 5.0 ml (from a 1M stock solution) HEPES, 0.1 ml TX-100, bring to 10 ml total volume with MilliQ H_2O.

e.   Lysis Buffer: 5.0 HEPES (from 1M stock solution.),
2.74 ml NaCl (from 5M stock solution), 10 ml glycerol, 1.0 ml
TX-100, 0.4 ml EDTA (from a 100 mM stock solution), 1.0 ml
PMSF (from a 100 mM stock solution), 0.1 ml Na_3VO_4 (from a 0.1

5   M stock solution), bring to 100 ml total volume with MilliQ
H_2O.

f.   ATP: Sigma Cat. # A-7699, make up 10 mM stock
solution (5.51 mg/ml).

g   TRIS-HCl: Fischer Cat. # BP 152-5, to 600 ml MilliQ

10 H_2O add 121.14 g material, adjust pH to 7.5 with HCl, bring to
1 L total volume with MilliQ H_2O.

h.   NaCl: Fischer Cat. # S271-10, Make up 5M stock
solution with MilliQ H_2O.

i. Na_3VO_4: Fischer Cat. # S454-50, to 80 ml MilliQ H_2O,

15 add 1.8 g material, adjust pH to 10.0 with HCl or NaOH, boil
in a microwave, cool, check pH, repeat pH adjustment until pH
remains stable after heating/cooling cycle, bring to 100 ml
total volume with MilliQ H_2O, make 1 ml aliquots and store at -
80°C.

20   j. $MgCl_2$: Fischer Cat. # M33-500, make up 1M stock
solution with MilliQ H_2O.

k.   HEPES: Fischer Cat. # BP 310-500, to 200 ml MilliQ
H_2O, add 59.6 g material, adjust pH to 7.5, bring to 250 ml
total volume with MilliQ H_2O, sterile filter (1M stock

25 solution).

l.   TBST Buffer: TBST Buffer: To 900 ml dH_2O add 6.057
g TRIS and 8.766 g NaCl, adjust pH to 7.2 with HCl, add 1.0 ml
Triton-X100, bring to 1 L total volume with dH_2O.

m. $MnCl_2$: Fischer Cat. # M87-100, make up 1M stock

30 solution with MilliQ H_2O.

n.   DTT: Fischer Cat. # BP172-5.

o.   TBS (TRIS Buffered Saline): to 900 ml MilliQ H_2O add
6.057 g TRIS and 8.777 g NaCl, bring to 1 L total volume with
MilliQ H_2O.

p.   Kinase Reaction Mixture: Amount per assay plate (100 wells): 1.0 ml Kinase Buffer, 200 µg GST-ζ , bring to final volume of 8.0 ml with MilliQ H_2O.

q.   Biotin labeled EEEYEEYEEEYEEEYEEEY: Make peptide stock solution (1mM, 2.98 mg/ml) in water fresh just before use.

r.   Vectastain ELITE ABC reagent: To prepare 14 ml of working reagent, add 1 drop of reagent A to 15 ml TBST and invert tube several times to mix. Then add 1 drop of reagent B. Put tube on orbital shaker at room temperature and mix for 30 minutes.

Procedures:

Preparation of src coated ELISA plate.

1.   Coat ELISA plate with 0.5 µg/well anti-src mab in 100 µl of pH 9.6 sodium carbonate buffer, hold at 4°C overnight.

2.   Wash wells once with PBS.

3.   Block plate with 0.15 ml 5% milk in PBS for 30 min. at room temperature.

4.   Wash plate 5X with PBS.

5.   Add 10 µg/well of src transformed yeast lysates diluted in Lysis Buffer (0.1 ml total volume per well). (Amount of lysate may vary between batches.) Shake plate for 20 minutes at room temperature.

Preparation of phosphotyrosine antibody-coated ELISA plate.

1.   4G10 plate: coat 0.5 µg/well 4G10 in 100 µl PBS overnight at 4°C and block with 150 µl of 5% milk in PBS for 30 minutes at room temperature.

Kinase assay procedure.

1.   Remove unbound proteins from plates and wash plates 5X with PBS.

2.   Add 0.08 ml Kinase Reaction Mixture per well (containing 10 µl of 10X Kinase Buffer and 10 µM (final concentration) biotin-EEEYEEYEEEYEEEYEEEY per well diluted in water.

5   3.   Add 10 µl of compound diluted in water containing 10% DMSO and pre-incubate for 15 minutes at room temperature.

4.   Start kinase reaction by adding 10 µl/well of 0.05 mM ATP in water (5 µM ATP final).

5.   Shake ELISA plate for 15 min. at room temperature.

10   6.   Stop kinase reaction by adding 10 µl of 0.5 M EDTA per well.

7.   Transfer 90 µl supernatant to a blocked 4G10 coated ELISA plate.

8.   Incubate for 30 min. while shaking at room

15 temperature.

9.   Wash plate 5X with TBST.

10. Incubate with Vectastain ELITE ABC reagent (100 µl/well) for 30 min. at room temperature.

11. Wash the wells 5X with TBST.

20   12. Develop with Turbo TMB.

Biochemical lck Assay

This assay is used to determine lck protein kinase activities measuring phosphorylation of GST-ζ as the readout.

Materials and Reagents:

25   a.   Yeast transformed with lck. Schizosaccharomyces Pombe is used to express recombinant Lck (Superti-Furga, et al., EMBO J, 12:2625-2634, Superti-Furga, et al., Nature Biotech., 14:600-605). S. Pombe strain SP200 (h-s leul.32 ura4 ade210) is grown as described and transformations with

30 pRSP expression plasmids are done by the lithium acetate method (Superti-Furga, supra). Cells are grown in the presence of 1 µM thiamine to induce expression.

      b.   Cell lysates: Yeast cells expressing lck are pelleted, washed once in water, re-pelleted and stored frozen at -80°C until use.

      c.   GST-ζ: DNA encoding for GST-ζ fusion protein for expression in bacteria obtained from Arthur Weiss of the Howard Hughes Medical Institute at the University of California, San Francisco. Transformed bacteria are grown overnight while shaking at 25°C. GST-ζ is purified by glutathione affinity chromatography, Pharmacia, Alameda, CA.

      d.   DMSO: Sigma, St. Louis, MO.

      e.   96-Well ELISA plate: Corning 96 Well Easy Wash, Modified Flat Bottom Plate, Corning Cat. #25805-96.

      f.   NUNC 96-well V-bottom polypropylene plates for dilution of compounds: Applied Scientific Cat. # AS-72092.

      g.   Purified Rabbit anti-GST antiserum: Amrad Corporation (Australia) Cat. #90001605.

      h.   Goat anti-Rabbit-IgG-HRP: Amersham Cat. # V010301.

      i.   Sheep ant-mouse IgG (H+L): Jackson Labs Cat. # 5215-005-003.

      j.   Anti-Lck (3A5) mab: Santa Cruz Biotechnology Cat # sc-433.

      k.   Monoclonal anti-phosphotyrosine UBI 05-321 (UB40 may be used instead).

Buffer solutions:

      a.   PBS (Dulbecco's Phosphate-Buffered Saline) 1X solution: GIBCO PBS, GIBCO Cat. # 450-1300EB.

      b.   Blocking Buffer: 100 g. BSA, 12.1 g. TRIS (pH7.5), 58.44 g NaCl, 10 ml Tween-20, bring up to 1 L total volume with MilliQ H_2O.

      c.   Carbonate Buffer: Na_2CO_4 from Fischer, Cat. # S495, make up 100 mM solution with MilliQ H_2O.

      d.   Kinase Buffer: 1.0 ml (from 1M stock solution) $MgCl_2$, 0.2 ml (from a 1M stock solution) $MnCl_2$, 0.2 ml (from a 1M

stock solution) DTT, 5.0 ml (from a 1M stock solution) HEPES, 0.1 ml TX-100, bring to 10 ml total volume with MilliQ H$_2$O.

e.   Lysis Buffer: 5.0 HEPES (from 1M stock solution.), 2.74 ml NaCl (from 5M stock solution), 10 ml glycerol, 1.0 ml TX-100, 0.4 ml EDTA (from a 100 mM stock solution), 1.0 ml PMSF (from a 100 mM stock solution), 0.1 ml Na$_3$VO$_4$ (from a 0.1 M stock solution), bring to 100 ml total volume with MilliQ H$_2$O.

f.   ATP: Sigma Cat. # A-7699, make up 10 mM stock solution (5.51 mg/ml).

g   TRIS-HCl: Fischer Cat. # BP 152-5, to 600 ml MilliQ H$_2$O add 121.14 g material, adjust pH to 7.5 with HCl, bring to 1 L total volume with MilliQ H$_2$O.

h.   NaCl: Fischer Cat. # S271-10, Make up 5M stock solution with MilliQ H$_2$O.

i   Na$_3$VO$_4$: Fischer Cat. # S454-50, to 80 ml MilliQ H$_2$O, add 1.8 g material, adjust pH to 10.0 with HCl or NaOH, boil in a microwave, cool, check pH, repeat pH adjustment until pH remains stable after heating/cooling cycle, bring to 100 ml total volume with MilliQ H$_2$O, make 1 ml aliquots and store at -80°C.

j.   MgCl$_2$: Fischer Cat. # M33-500, make up 1M stock solution with MilliQ H$_2$O.

k.   HEPES: Fischer Cat. # BP 310-500, to 200 ml MilliQ H$_2$O, add 59.6 g material, adjust pH to 7.5, bring to 250 ml total volume with MilliQ H$_2$O, sterile filter (1M stock solution).

l.   Albumin, Bovine (BSA), Sigma Cat. # A4503, to 150 ml MilliQ H$_2$O add 30 g material, bring 300 ml total volume with MilliQ H$_2$O, filter through 0.22 µm filter, store at 4°C.

m.   TBST Buffer: To 900 ml dH$_2$O add 6.057 g TRIS and 8.766 g NaCl, adjust pH to 7.2 with HCl, add 1.0 ml Triton-X100, bring to 1 L total volume with dH$_2$O.

n.   MnCl2:   Fischer Cat. # M87-100, make up 1M stock solution with MilliQ H$_2$O.

o.   DTT: Fischer Cat. # BP172-5.

p.   TBS (TRIS Buffered Saline): to 900 ml MilliQ H$_2$O add

5   6.057 g TRIS and 8.777 g NaCl, bring to 1 L total volume with MilliQ H$_2$O.

q   Kinase Reaction Mixture: Amount per assay plate (100 wells): 1.0 ml Kinase Buffer, 200 μg GST-ζ, bring to final volume of 8.0 ml with MilliQ H$_2$O.

10   **Procedures:**

Preparation of Lck coated ELISA plate.

1.   Coat 2.0 μg/well Sheep anti-mouse IgG in 100 μl of pH 9.6 sodium carbonate buffer at 4°C overnight.

2.   Wash well once with PBS.

15   3.   Block plate with 0.15 ml of blocking Buffer for 30 min. at room temp.

4.   Wash plate 5X with PBS.

5.   Add 0.5 μg/well of anti-lck (mab 3A5) in 0.1 ml PBS at room temperature for 1-2 hours.

20   6.   Wash plate 5X with PBS.

7.   Add 20 μg/well of lck transformed yeast lysates diluted in Lysis Buffer (0.1 ml total volume per well). Shake plate at 4°C overnight to prevent loss of activity.

Preparation of phosphotyrosine antibody-coated ELISA

25   **plate.**

1.   UB40 plate: 1.0 μg/well UB40 in 100 μl of PBS overnight at 4°C and block with 150 μl of Blocking Buffer for at least 1 hour.

Kinase assay procedure.

30   1.   Remove unbound proteins from plates and wash plates 5X with PBS.

2.   Add 0.08 ml Kinase Reaction Mixture per well
(containing 10 µl of 10X Kinase Buffer and 2 µg GST-ζ per well
diluted with water).

3.   Add 10 µl of compound diluted in water containing
10% DMSO and pre-incubate for 15 minutes at room temperature.

4.   Start kinase reaction by adding 10µl/well of 0.1 mM
ATP in water (10 µM ATP final).

5.   Shake ELISA plate for 60 min. at room temperature.

6.   Stop kinase reaction by adding 10 µl of 0.5 M EDTA
per well.

7.   Transfer 90 µl supernatant to a blocked 4G10 coated
ELISA plate from section B, above.

8.   Incubate while shaking for 30 min. at room
temperature.

9.   Wash plate 5X with TBST.

10.   Incubate with Rabbit anti-GST antibody at 1:5000
dilution in 100 µl TBST for 30 min. at room temperature.

11.   Wash the wells 5X with TBST.

12.   Incubate with Goat anti-Rabbit-IgG-HRP at 1:20,000
dilution in 100 µl of TBST for 30 min. at room temperature.

13.   Wash the wells 5X with TBST.

14.   Develop with Turbo TMB.

Assay measuring phosphorylating function of RAF.

The following assay reports the amount of RAF-catalyzed
phosphorylation of its target protein MEK as well as MEK's
target MAPK. The RAF gene sequence is described in Bonner et
al., 1985, <u>Molec. Cell. Biol.</u>, 5:1400-1407, and is readily
accessible in multiple gene sequence data banks. Construction
of the nucleic acid vector and cell lines utilized for this
portion of the invention are fully described in Morrison et
al., 1988, <u>Proc. Natl. Acad. Sci. USA</u>, 85:8855-8859.

Materials and Reagents

1.   Sf9 (Spodoptera frugiperda) cells, GIBCO-BRL, Gaithersburg, MD.

2.   RIPA buffer: 20 mM Tris/HCl pH 7.4, 137 mM NaCl, 10% glycerol, 1 mM PMSF, 5 mg/L Aprotenin, 0.5 % Triton X-100,

3.   Thioredoxin-MEK fusion protein (T-MEK): T-MEK expression and purification by affinity chromatography are performed according to the manufacturer's procedures. Catalog# K 350-01 and R 350-40, Invitrogen Corp., San Diego, CA

4.   His-MAPK (ERK 2), His-tagged MAPK is expressed in XL1 Blue cells transformed with pUC18 vector encoding His-MAPK.   His-MAPK is purified by Ni-affinity chromatography. Cat# 27-4949-01, Pharmacia, Alameda, CA, as described herein.

5.   Sheep anti mouse IgG: Jackson laboratories, West Grove, PA.   Catalog, # 515-006-008, Lot# 28563

6.   RAF-1 protein kinase specific antibody: URP2653 from UBI.

7.   Coating buffer: PBS, phosphate buffered saline, GIBCO-BRL, Gaithersburg, MD.

8.   Wash buffer: TBST (50 mM Tris/HCL pH 7.2, 150 mM NaCl, 0.1 % Triton X-100).

9.   Block buffer: TBST, 0.1 % ethanolamine pH 7.4

10.   DMSO, Sigma, St. Louis, MO

11.   Kinase buffer (KB): 20 mM HEPES/HCl pH 7.2, 150 mM NaCl, 0.1 % Triton X-100, 1 mM PMSF, 5 mg/L Aprotenin, 75 mM sodium orthovanadate, 0.5 MM DTT and 10 mM $MgCl_2$.

12.   ATP mix: 100 mM $MgCl_2$, 300 mM ATP, 10 mCi $\gamma^{33}P$ ATP (Dupont-NEN)/mL.

13   Stop solution: 1% phosphoric acid, Fisher, Pittsburgh, PA.

14.   Wallac Cellulose Phosphate Filter mats, Wallac, Turku, Finnland.

15. Filter wash solution: 1% phosphoric acid, Fisher, Pittsburgh, PA.

16. Tomtec plate harvester, Wallac, Turku, Finnland.

17. Wallac beta plate reader # 1205, Wallac, Turku, Finnland.

18. NUNC 96-well V bottom polypropylene plates for compounds Applied Scientific Catalog # AS-72092.

Procedure

All of the following steps are conducted at room temperature unless specifically indicated otherwise.

1. ELISA plate coating: ELISA wells are coated with 100 ml of Sheep anti mouse affinity purified antiserum (1 mg/100 mL coating buffer) over night at 4° C. ELISA plates can be used for two weeks when stored at 4° C.

2. Invert the plate and remove liquid. Add 100 mL of blocking solution and incubate for 30 min.

3. Remove blocking solution and wash four times with wash buffer. Pat the plate on a paper towel to remove excess liquid.

4. Add 1 mg of antibody specific for RAF-1 to each well and incubate for 1 hour. Wash as described in step 3.

5. Thaw lysates from RAS/RAF infected Sf9 cells and dilute with TBST to 10 mg/100 mL. Add 10 mg of diluted lysate to the wells and incubate for 1 hour. Shake the plate during incubation. Negative controls receive no lysate. Lysates from RAS/RAF infected Sf9 insect cells are prepared after cells are infected with recombinant baculoviruses at a MOI of 5 for each virus, and harvested 48 hours later. The cells are washed once with PBS and lysed in RIPA buffer. Insoluble material is removed by centrifugation (5 min at 10,000 x g). Aliquots of lysates are frozen in dry ice/ethanol and stored at -80 °C until use.

6.   Remove non-bound material and wash as outlined above (step 3).

7.   Add 2 mg of T-MEK and 2 mg of His-MAEPK per well and adjust the volume to 40 ml with kinase buffer. Methods for purifying T-MEK and MAPK from cell extracts are provided herein by example.

8.   Pre-dilute compounds (stock solution 10 mg/ml DMSO) or extracts 20 fold in TBST plus 1% DMSO. Add 5 ml of the pre-diluted compounds/extracts to the wells described in step 6. Incubate for 20 min. Controls receive no drug.

9.   Start the kinase reaction by addition of 5 ml ATP mix, Shake the plates on an ELISA plate shaker during incubation.

10.   Stop the kinase reaction after 60 min by addition of 30 mL stop solution to each well.

11.   Place the phosphocellulose mat and the ELISA plate in the Tomtec plate harvester. Harvest and wash the filter with the filter wash solution according to the manufacturer's recommendation. Dry the filter mats. Seal the filter mats and place them in the holder. Insert the holder into radioactive detection apparatus and quantify the radioactive phosphorous on the filter mats.

Alternatively, 40 mL aliquots from individual wells of the assay plate can be transferred to the corresponding positions on the phosphocellulose filter mat. After air drying the filters, put the filters in a tray. Gently rock the tray, changing the wash solution at 15 min intervals for 1 hour. Air-dry the filter mats. Seal the filter mats and place them in a holder suitable for measuring the radioactive phosphorous in the samples. Insert the holder into a detection device and quantify the radioactive phosphorous on the filter mats.

CDK2/Cyclin A - Inhibition Assay

This assay analyzes the protein kinase activity of CDK2 in exogenous substrate.

Reagents:

5   A.   Buffer A: (80 mM Tris (pH 7.2), 40 mM $MgCl_2$), 4.84 g. Tris (F.W. =121.1 g/mol), 4.07 g. $MgCl_2$ (F.W.=203.31 g/mol) dissolved in 500 ml H_2O. Adjust pH to 7.2 with HCl.

B.   Histone H1 solution (0.45 mg/ml Histone H1 and 20 mM HEPES pH 7.2: 5 mg Histone H1 (Boehinger Mannheim) in 11.111

10   ml 20 mM HEPES pH 7.2 (477 mg HEPES (F.W.= 238.3 g/mol) dissolved in 100 ml ddH_2O, stored in 1 ml aliquots at -80° C.

C.   ATP solution (60 µM ATP, 300 µg/ml BSA, 3 mM DTT): 120 µl 10 mM ATP, 600 µl 10 mg/ml BSA to 20 ml, stored in 1 ml aliquots at -80° C.

15   D.   CDK2 solution: cdk2/cyclin A in 10 mM HEPES pH 7.2, 25 mM NaCl, o.5 mM DTT, 10% glycerol, stored in 9 µl aliquots at

-80° C.

Protocol

20   1.   Prepare solutions of inhibitors at three times the desired final assay concentration in ddH_2O/15% DMSO by volume.

2.   Dispense 20 µl of inhibitors to wells of polypropylene 96-well plates (or 20 µl 15% DMSO for positive and negative controls).

25   3.   Thaw Histone H1 solution (1 ml/plate), ATP solution (1 ml/plate plus 1 aliquot for negative control), and CDK2 solution (9 µl/plate). Keep CDK2 on ice until use. Aliquot CDK2 solution appropriately to avoid repeated freeze-thaw cycles.

30   4.   Dilute 9 µl CDK2 solution into 2.1 ml Buffer A (per plate). Mix. Dispense 20 µl into each well.

5.   Mix 1 ml Histone H1 solution with 1 ml ATP solution
(per plate) into a 10 ml screw cap tube. Add $\gamma^{33}P$ ATP to a
concentration of 0.15 µCi/20µl (0.15 µCi/well in assay). Mix
carefully to avoid BSA frothing. Add 20 µl to appropriate

5   wells. Mix plates on plate shaker. For negative control, mix
ATP solution with an equal amount of 20 mM HEPES pH 7.2 and
add $\gamma^{33}P$ ATP to a concentration of 0.15 µCi/20µl solution. Add
20 µl to appropriate wells.

6.   Let reactions proceed for 60 minutes.

10   7.   Add 35 µl 10% TCA to each well. Mix plates on plate
shaker.

8.   Spot 40 µl of each sample onto P30 filter mat
squares. Allow mats to dry (approx. 10-20 minutes).

9   Wash filter mats 4 X 10 minutes with 250 ml 1%

15   phosphoric acid (10 ml phosphoric acid per liter ddH$_2$O).

10.   Count filter mats with beta plate reader.

Cellular/Biologic Assays

PDGF-Induced BrdU Incorporation Assay

Materials and Reagents:

20   (1)   PDGF: human PDGF B/B, 1276-956, Boehringer Mannheim,
Germany.

(2)   BrdU Labeling Reagent: 10 mM, in PBS (pH7.4), Cat.
No. 1 647 229, Boehringer Mannheim, Germany.

(3)   FixDenat: fixation solution (ready to use), Cat. No.

25   1 647 229, Boehringer Mannheim, Germany.

(4)   Anti-BrdU-POD: mouse monoclonal antibody conjugated
with peroxidase, Cat. No. 1 647 229, Boehringer Mannheim,
Germany.

(5)   TMB Substrate Solution: tetramethylbenzidine (TMB),

30   ready to use, Cat. No. 1 647 229, Boehringer Mannheim,
Germany.

(6)   PBS Washing Solution : 1X PBS, pH 7.4 (Sugen, Inc.,
Redwood City, California).

(7)   Albumin, Bovine (BSA): fraction V powder, A-8551, Sigma Chemical Co., USA.

(8)   3T3 cell line genetically engineered to express human PDGF-R.

Protocol

(1)   Cells are seeded at 8000 cells/well in DMEM, 10% CS, 2mM Gln in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO_2.

(2)   After 24 hours, the cells are washed with PBS, and then are serum starved in serum free medium (0%CS DMEM with 0.1% BSA) for 24 hours.

(3)   On day 3, ligand (PDGF, 3.8 nM, prepared in DMEM with 0.1% BSA) and test compounds are added to the cells simultaneously. The negative control wells receive serum free DMEM with 0.1% BSA only, the positive control cells receive the ligand (PDGF) but no test compound. Test compounds are prepared in serum free DMEM with ligand in a 96 well plate, and serially diluted for 7 test concentrations.

(4)   After 20 hours of ligand activation, diluted BrdU labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the cells are incubated with BrdU (final concentration=10 μM) for 1.5 hours.

(5)   After incubation with labeling reagent, the medium is removed by decanting and tapping the inverted plate on a paper towel. FixDenat solution is added (50 μl/well) and the plates are incubated at room temperature for 45 minutes on a plate shaker.

(6)   The FixDenat solution is thoroughly removed by decanting and tapping the inverted plate on a paper towel. Milk is added (5% dehydrated milk in PBS, 200 μl/well) as a blocking solution and the plate is incubated for 30 minutes at room temperature on a plate shaker.

(7)   The blocking solution is removed by decanting and

the wells are washed once with PBS. Anti-BrdU-POD solution
(1:100 dilution in PBS, 1% BSA) is added (100 μl/well) and the
plate is incubated for 90 minutes at room temperature on a
plate shaker.

5   (8)   The antibody conjugate is thoroughly removed by
decanting and rinsing the wells 5 times with PBS, and the
plate is dried by inverting and tapping on a paper towel.

           (9)   TMB substrate solution is added (100 μl/well) and
incubated for 20 minutes at room temperature on a plate shaker
10   until color development is sufficient for photometric
detection.

           (10) The absorbance of the samples are measured at 410 nm
(in "dual wavelength" mode with a filter reading at 490 nm, as
a reference wavelength) on a Dynatech ELISA plate reader.

15   **EGF-Induced BrdU Incorporation Assay**

           Materials and Reagents

           (1)   EGF: mouse EGF, 201, Toyobo, Co., Ltd. Japan.

           (2)   BrdU Labeling Reagent: 10 mM, in PBS (pH7.4), Cat.
No. 1 647 229, Boehringer Mannheim, Germany.

20   (3)   FixDenat: fixation solution (ready to use), Cat. No.
1 647 229, Boehringer Mannheim, Germany.

           (4)   Anti-BrdU-POD: mouse monoclonal antibody conjugated
with peroxidase, Cat. No. 1 647 229, Boehringer Mannheim,
Germany.

25   (5)   TMB Substrate Solution: tetramethylbenzidine (TMB),
ready to use, Cat. No. 1 647 229, Boehringer Mannheim,
Germany.

           (6)   PBS Washing Solution : 1X PBS, pH 7.4 (Sugen, Inc.,
Redwood City, California).

30   (7)   Albumin, Bovine (BSA): fraction V powder, A-8551,
Sigma Chemical Co., USA.

           (8)   3T3 cell line genetically engineered to express
human EGF-R.

Protocol

(1)   Cells are seeded at 8000 cells/well in 10% CS, 2mM Gln in DMEM, in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO_2.

5   (2)   After 24 hours, the cells are washed with PBS, and then are serum starved in serum free medium (0% CS DMEM with 0.1% BSA) for 24 hours.

(3)   On day 3, ligand (EGF, 2 nM, prepared in DMEM with 0.1% BSA) and test compounds are added to the cells

10   simultaneously.   The negative control wells receive serum free DMEM with 0.1% BSA only, the positive control cells receive the ligand (EGF) but no test compound.   Test compounds are prepared in serum free DMEM with ligand in a 96 well plate, and serially diluted for 7 test concentrations.

15   (4)   After 20 hours of ligand activation, diluted BrdU labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the cells are incubated with BrdU (final concentration = 10 μM) for 1.5 hours.

(5)   After incubation with labeling reagent, the medium

20   is removed by decanting and tapping the inverted plate on a paper towel.   FixDenat solution is added (50 μl/well) and the plates are incubated at room temperature for 45 minutes on a plate shaker.

(6)   The FixDenat solution is thoroughly removed by

25   decanting and tapping the inverted plate on a paper towel. Milk is added (5% dehydrated milk in PBS, 200 μl/well) as a blocking solution and the plate is incubated for 30 minutes at room temperature on a plate shaker.

(7)   The blocking solution is removed by decanting and

30   the wells are washed once with PBS.   Anti-BrdU-POD solution (1:100 dilution in PBS, 1% BSA) is added   (100 μl/well) and the plate is incubated for 90 minutes at room temperature on a plate shaker.

(8)   The antibody conjugate is thoroughly removed by decanting and rinsing the wells 5 times with PBS, and the plate is dried by inverting and tapping on a paper towel.

(9)   TMB substrate solution is added (100 µl/well) and

5   incubated for 20 minutes at room temperature on a plate shaker until color development is sufficient for photometric detection.

(10) The absorbance of the samples are measured at 410 nm (in "dual wavelength" mode with a filter reading at 490 nm, as

10   a reference wavelength) on a Dynatech ELISA plate reader.

EGF-Induced Her2-Driven BrdU Incorporation

Materials and Reagents

(1)   EGF: mouse EGF, 201, Toyobo, Co., Ltd. Japan

(2)   BrdU Labeling Reagent: 10 mM, in PBS (pH7.4), Cat.

15   No. 1 647 229, Boehringer Mannheim, Germany.

(3)   FixDenat: fixation solution (ready to use), Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(4)   Anti-BrdU-POD: mouse monoclonal antibody conjugated with peroxidase, Cat. No. 1 647 229, Boehringer Mannheim,

20   Germany.

(5)   TMB Substrate Solution: tetramethylbenzidine (TMB), ready to use, Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(6)   PBS Washing Solution: 1X PBS, pH 7.4, made in house.

25   (7)   Albumin, Bovine (BSA): fraction V powder, A-8551, Sigma Chemical Co., USA.

(8)   3T3 cell line engineered to express a chimeric receptor having the extra-cellular domain of EGF-R and the intra-cellular domain of Her2.

30   **Protocol**

(1)   Cells are seeded at 8000 cells/well in DMEM, 10% CS, 2mM Gln in a 96- well plate. Cells are incubated overnight at 37° C in 5% CO_2.

(2)   After 24 hours, the cells are washed with PBS, and then are serum starved in serum free medium (0% CS DMEM with 0.1% BSA) for 24 hours.

(3)   On day 3, ligand (EGF = 2 nM, prepared in DMEM with

5   0.1% BSA) and test compounds are added to the cells simultaneously. The negative control wells receive serum free DMEM with 0.1% BSA only, the positive control cells receive the ligand (EGF) but no test compound. Test compounds are prepared in serum free DMEM with ligand in a 96 well plate,

10   and serially diluted for 7 test concentrations.

(4)   After 20 hours of ligand activation, diluted BrdU labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the cells are incubated with BrdU (final concentration = 10 μM) for 1.5 hours.

15   (5)   After incubation with labeling reagent, the medium is removed by decanting and tapping the inverted plate on a paper towel. FixDenat solution is added (50 μl/well) and the plates are incubated at room temperature for 45 minutes on a plate shaker.

20   (6)   The FixDenat solution is thoroughly removed by decanting and tapping the inverted plate on a paper towel. Milk is added (5% dehydrated milk in PBS, 200 μl/well) as a blocking solution and the plate is incubated for 30 minutes at room temperature on a plate shaker.

25   (7)   The blocking solution is removed by decanting and the wells are washed once with PBS. Anti-BrdU-POD solution (1:100 dilution in PBS, 1% BSA) is added (100 μl/well) and the plate is incubated for 90 minutes at room temperature on a plate shaker.

30   (8)   The antibody conjugate is thoroughly removed by decanting and rinsing the wells 5 times with PBS, and the plate is dried by inverting and tapping on a paper towel.

(9) TMB substrate solution is added (100 µl/well) and incubated for 20 minutes at room temperature on a plate shaker until color development is sufficient for photometric detection.

(10) The absorbance of the samples are measured at 410 nm (in "dual wavelength" mode with a filter reading at 490 nm, as a reference wavelength) on a Dynatech ELISA plate reader.

IGF1-Induced BrdU Incorporation Assay

Materials and Reagents

(1) IGF1 Ligand: human, recombinant, G511, Promega Corp, USA.

(2) BrdU Labeling Reagent: 10 mM, in PBS (pH7.4), Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(3) FixDenat: fixation solution (ready to use), Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(4) Anti-BrdU-POD: mouse monoclonal antibody conjugated with peroxidase, Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(5) TMB Substrate Solution: tetramethylbenzidine (TMB), ready to use, Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(6) PBS Washing Solution: 1X PBS, pH 7.4 (Sugen, Inc., Redwood City, California).

(7) Albumin, Bovine (BSA): fraction V powder, A-8551, Sigma Chemical Co., USA.

(8) 3T3 cell line genetically engineered to express human IGF-1 receptor.

Protocol

(1) Cells are seeded at 8000 cells/well in DMEM, 10% CS, 2mM Gln in a 96- well plate. Cells are incubated overnight at 37°C in 5% CO_2.

(2)   After 24 hours, the cells are washed with PBS, and
then are serum starved in serum free medium (0%CS DMEM with
0.1% BSA) for 24 hours.

(3)   On day 3, ligand (IGF1 = 3.3 nM, prepared in DMEM

5   with 0.1% BSA) and test compounds are added to the cells
simultaneously. The negative control wells receive serum free
DMEM with 0.1% BSA only, the positive control cells receive
the ligand (IGF1) but no test compound. Test compounds are
prepared in serum free DMEM with ligand in a 96 well plate,

10   and serially diluted for 7 test concentrations.

(4)   After 16 hours of ligand activation, diluted BrdU
labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the
cells are incubated with BrdU (final concentration=10 μM) for
1.5 hours.

15   (5)   After incubation with labeling reagent, the medium
is removed by decanting and tapping the inverted plate on a
paper towel. FixDenat solution is added (50 μl/well) and the
plates are incubated at room temperature for 45 minutes on a
plate shaker.

20   (6)   The FixDenat solution is thoroughly removed by
decanting and tapping the inverted plate on a paper towel.
Milk is added (5% dehydrated milk in PBS, 200 μl/well) as a
blocking solution and the plate is incubated for 30 minutes at
room temperature on a plate shaker.

25   (7)   The blocking solution is removed by decanting and
the wells are washed once with PBS. Anti-BrdU-POD solution
(1:100 dilution in PBS, 1% BSA) is added (100 μl/well) and the
plate is incubated for 90 minutes at room temperature on a
plate shaker.

30   (8)   The antibody conjugate is thoroughly removed by
decanting and rinsing the wells 5 times with PBS, and the
plate is dried by inverting and tapping on a paper towel.

(9) TMB substrate solution is added (100 µl/well) and
incubated for 20 minutes at room temperature on a plate shaker
until color development is sufficient for photometric
detection.

5   (10) The absorbance of the samples are measured at 410 nm
(in "dual wavelength" mode with a filter reading at 490 nm, as
a reference wavelength) on a Dynatech ELISA plate reader.

FGF-Induced BrdU incorporation Assay

This assay measures FGF-induced DNA synthesis in
10   3Tc7/EGFr cells that express endogenous FGF receptors.

Materials and Reagents:

1.   FGF: human FGF2/bFGF (Gibco BRL, No. 13256-029).

2.   BrdU Labeling reagent, (10 mM PBS (pH 7.4),
Boehringer Mannheim Cat No. 1 647 229).

15   3.   Fixdenat fixation solution (Boehringer Mannheim Cat
No. 1 647 229).

4.   Anti-BrdU-POD (mouse monoclonal antibody conjugated
with peroxidase, Boehringer Mannheim Cat. No. 1 647 229).

5.   TMB (tetramethylbenzidine, Boehringer Mannheim Cat.
20   No. 1 647 229).

6.   PBS washing solution, pH 7.4 (Sugen, Inc.).

7.   Albumin, bovine (BSA), fraction V powder (Sigma
Chemical Co., Cat. No. A-8551)

Procedure

25   1.   3T3 engineered cell line: 3T3c7/EGFr.

2.   Cells are seeded at 8,000 cells/well in DMEM, 10% CS
and 2 mM Gln in a 96-well plate. Incubate 24 hours at 37° C in
5% CO_2.

3.   After 24 hours, wash cells with PBS then serum
30   starve in serum free medium (0% DMEM, 0.1% BSA) for 24 hours.

4.   Add ligand (FGF2 (1.5 nM in DMEM with 0.1% BSA) and
test compound simultaneously. Negative control wells receive
serum free DMEM with 0.1% BSA only, positive control wells

receive FGF2 ligand but no test compound. Test compounds are prepared in serum-free DMEM with ligand in a 96-well plate and serially diluted to make seven (7) test concentrations.

5.   After 20 hours, add diluted BrdU labeling reagent
(1:100 BrdU:DMEM, 0.1% BSA, final concentration is 10 µM) to the cells and incubate for 1.5 hours.

6.   Decant medium. Remove traces of material with paper towel. Add FixDenat (50 µl/well) and incubate at room temperature for 45 minutes on a plate shaker.

7.   Remove Fixdenat solution. Add blocking solution (5% dehydrated milk in PBS (200 µl/well)) and incubate for 30 minutes at room temperature on a plate shaker.

8.   Decant blocking solution, wash wells once with PBS. Add anti-BrdU-POD solution (1:100 dilution in PBS, 0.1% BSA), incubate for 90 minutes at room temperature on a plate shaker.

9.   Decant antibody conjugate, rinse wells 5 times with PBS. Dry plate by inverting on paper towel and tapping.

10.   Add TMB solution (100 µl/well), incubate 20 minutes at room temperature on a plate shaker until color development is sufficient for photometric detection.

11.   Measure absorbance at 410 nM on a Dynatech ELISA plate reader using "Dual wavelength" mode with a filter at 490 nM.

Biochemical EGFR Assay

This assay measures the _in vitro_ kinase activity of EGFR using ELISA.

Materials and Reagents

1.   Corning 96-well Elisa plates (Corning Catalog No. 25805-96).

2.   SUMO1 monoclonal anti-EGFR antibody (Biochemistry Lab, SUGEN, Inc.).

3.   PBS (Dulbecco's Phosphate-Buffered Saline, Gibco Catalog No. 450-1300EB).

4.   TBST Buffer

Reagent	M.W.	Working Concentration	Amount per L
Tris	121.14	50 mM	6.057 g
NaCl	58.44	150 mM	8.766 g
Triton X-100	NA	0.1%	1.0 ml

5.   Blocking Buffer:

Reagent	M.W.	Working Concentration	Amount per 100 ml
Carnation Instant Non-Fat Milk		5%	5.0 g
PBS	NA	NA	100 ml

6.   A431 cell lysate (Screening Lab, SUGEN, Inc.)

7.   TBS Buffer:

Reagent	M.W.	Working Concentration	Amount per L
Tris	121.14	50 mM	6.057 g
NaCl	58.44	150 mM	8.766 g

8.   TBS + 10% DMSO

Reagent	M.W.	Working Concentration	Amount per L
Tris	121.14	50 mM	1.514 g
NaCl	58.44	150 mM	2.192 g
DMSO	NA	10%	25 ml

9.   Adenosine-5'-triphosphate (ATP, from Equine muscle, Sigma Cat. No. A-5394).

Prepare a 1.0 mM solution in dH_2O. This reagent should be made up immediately prior to use and kept on ice.

10. $MnCl_2$.

Prepare a 1.0 M stock solution in dH_2O.

11.   ATP/$MnCl_2$ phosphorylation mix

Reagent	Stock solution	Amount per 10 ml	Working Concentration

ATP	1.0 mM	300 μl	30 μM
MnCl$_2$	1.0 M	500 μl	50 mM
dH$_2$O			9.2 ml

This reagent should be prepared immediately before

5   use and kept on ice.

12.   NUNC 96-well V bottom polypropylene plates (Applied Scientific Cat. No. AS-72092).

13.   Ethylenediaminetetraacetic acid (EDTA)

Prepare 200 mM working solution in dH$_2$O. Adjust to pH

10   8.0 with 10 N NaOH.

14.   Rabbit polyclonal anti-phosphotyrosine serum (Biochemistry Lab, SUGEN, Inc.)

15.   Goat anti-rabbit IgG peroxidase conjugate (Biosource Cat. No. ALI0404)

15   16.   ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), Sigma Cat. No. A-1888).

Reagent	M.W.	Working Concentration	Amount per L
Citric Acid	192.12	100 mM	19.21 g
Na2HP04	141.96	250 mM	35.49 g
ABTS	NA	0.5 mg/ml	500 mg

Mix first two ingredients in about 900 ml dH$_2$O, adjust pH to 4.0 with phosphoric acid. Add ABTS, cover, let sit about 0.5 hr., filter. The solution should be kept in the dark at

25   4° C until ready to use.

17.   Hydrogen peroxide 30% solution (Fisher Cat. No. H325)

18.   ABTS/H$_2$O$_2$

Mix 15 ml ABTS solution and 2.0 μl H$_2$O$_2$. Prepare 5

30   minutes before use.

19.   0.2 M HCl

Procedure

1.   Coat Corning 96 well ELISA plates with 0.5 µg SUMO1 in 100 µl PBS per well, store overnight at 4° C.

2.   Remove unbound SUMO1 from wells by inverting plate to remove liquid. Wash 1x with dH$_2$O. Pat the plate on a paper towel to remove excess liquid.

3.   Add 150 µl of Blocking Buffer to each well. Incubate for 30 min. at room temperature with shaking.

4.   Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

5.   Dilute lysate in PBS (7 µg lysate/100 µl PBS).

6.   Add 100 µl of diluted lysate to each well. Shake at room temperature for 60 min.

7.   Wash plates as described in 4, above.

8.   Add 120 µl TBS to ELISA plate containing captured EGFR.

9.   Dilute test compound 1:10 in TBS in 96-well polypropylene plates (ie. 10 µl compound + 90 µl TBS).

10.   Add 13.5 µl diluted test compound to ELISA plate. To control wells (wells which do not receive any test compound), add 13.5 µl TBS + 10% DMSO.

11.   Incubate for 30 minutes while shaking at room temperature.

12.   Add 15 µl phosphorylation mix directly to all wells except negative control well which does not receive ATP/MnCl$_2$ (final well volume should be approximately 150 µl with 3 µM ATP/5 mM MnCl$_2$ final concentration in each well.) Incubate 5 minutes while shaking.

13.   After 5 minutes, stop reaction by adding 16.5 µl of 200 mM EDTA (pH 8.0) to each well, shaking continuously. After the EDTA has been added, shake for 1 min.

14.   Wash 4x with deionized water, twice with TBST.

15.   Add 100 μl anti-phosphotyrosine (1:3000 dilution in TBST) per well.   Incubate 30-45 min. at room temperature, with shaking.

16.   Wash as described in 4, above.

17.   Add 100 μl Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well.   Incubate 30 min. at room temperature, with shaking.

18.   Wash as described in 4, above.

19.   Add 100 μl of ABTS/H_2O_2 solution to each well.

20.   Incubate 5 to 10 minutes with shaking. Remove any bubbles.

21.   If necessary stop reaction with the addition of 100 μl 0.2 M HCl per well.

22.   Read assay on Dynatech MR7000 ELISA reader. Test Filter: 410 nM Reference Filter: 630 Nm.

Biochemical PDGFR Assay

This assay measures the _in vitro_ kinase activity of PDGFR using ELISA.

Materials and Reagents

Unless otherwise noted, the preparation of working solution of the following reagents is the same as that for the Biochemical EGFR assay, above.

1.   Corning 96-well Elisa plates (Corning Catalog No. 25805-96).

2.   28D4C10 monoclonal anti-PDGFR antibody (Biochemistry Lab, SUGEN, Inc.).

3.   PBS (Dulbecco's Phosphate-Buffered Saline, Gibco Catalog No. 450-1300EB)

4.   TBST Buffer.

5.   Blocking Buffer.

6.   PDGFR-β expressing NIH 3T3 cell lysate (Screening Lab, SUGEN, Inc.).

7.   TBS Buffer.

8.   TBS + 10% DMSO.

9.   Adenosine-5'-triphosphate (ATP, from Equine muscle, Sigma Cat. No. A-5394).

10. $MnCl_2$.

11.   Kinase buffer phosphorylation mix.

Reagent	Stock solution	Amount per 10 ml	Working Concentration
Tris	1 M	250 µl	25 mM
NaCl	5 M	200 µl	100 mM
$MnCl_2$	1 M	100 µl	10 mM
TX-100	100 mM	50 µl	0.5 mM

12.   NUNC 96-well V bottom polypropylene plates (Applied Scientific Cat. No. AS-72092).

13.   Ethylenediaminetetraacetic acid (EDTA).

14.   Rabbit polyclonal anti-phosphotyrosine serum (Biochemistry Lab, SUGEN, Inc.).

15.   Goat anti-rabbit IgG peroxidase conjugate (Biosource Cat. No. ALI0404).

16.   2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, Sigma Cat. No. A-1888).

17.   Hydrogen peroxide 30% solution (Fisher Cat. No. H325).

18.   ABTS/H_2O_2.

19.   0.2 M HCl.

Procedure

1.   Coat Corning 96 well ELISA plates with 0.5 µg 28D4C10 in 100 µl PBS per well, store overnight at 4° C.

2.   Remove unbound 28D4C10 from wells by inverting plate to remove liquid. Wash 1x with dH_2O. Pat the plate on a paper towel to remove excess liquid.

3.   Add 150 µl of Blocking Buffer to each well. Incubate for 30 min. at room temperature with shaking.

4.   Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

5.   Dilute lysate in HNTG (10 μg lysate/100 μl HNTG)

6.   Add 100 μl of diluted lysate to each well. Shake at room temperature for 60 min.

7.   Wash plates as described in 4, above.

8.   Add 80 μl working kinase buffer mix to ELISA plate containing captured PDGFR.

9.   Dilute test compound 1:10 in TBS in 96-well polypropylene plates (i.e., 10 μl compound + 90 μl TBS).

10.   Add 10 μl diluted test compound to ELISA plate. To control wells (wells which do not receive any test compound), add 10 μl TBS + 10% DMSO.

11.   Incubate for 30 minutes while shaking at room temperature.

12.   Add 10 μl ATP directly to all wells except negative control well (final well volume should be approximately 100 μl with 20 μM ATP in each well.) Incubate 30 minutes while shaking.

13.   After 30 minutes, stop reaction by adding 10 μl of 200 mM EDTA (pH 8.0) to each well.

14.   Wash 4x with deionized water, twice with TBST.

15.   Add 100 μl anti-phosphotyrosine (1:3000 dilution in TBST) per well. Incubate 30-45 min. at room temperature, with shaking.

16.   Wash as described in 4, above.

17.   Add 100 μl Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well. Incubate 30 min. at room temperature, with shaking.

18.   Wash as described in 4, above.

19.   Add 100 μl of ABTS/H_2O_2 solution to each well.

20.   Incubate 10 to 30 minutes with shaking. Remove any bubbles.

21.   If necessary stop reaction with the addition of 100 µl 0.2 M HCl per well.

22.   Read assay on Dynatech MR7000 ELISA reader: test filter: 410 nM, reference filter: 630 nM.

Biochemical FGFR Assay

This assay measures in vitro kinase activity of the Myc-GyrB-FGFR fusion protein using ELISA.

Materials and Reagents

1. HNTG

Reagent	M.W.	5x Stock Concentration	Amount per L	1x Working Concentration
HEPES	238.3	100 mM	23.83 g	20 mM
NaCl	58.44	750 mM	43.83 g	150 mM
Glycerol	NA	50%	500 ml	10%
Triton X-100	NA	5%	10 ml	1.0%

To make a liter of 5x stock solution, dissolve HEPES and NaCl in about 350 ml dH$_2$O, adjust pH to 7.2 with HCl or NaOH (depending on the HEPES that is used), add glycerol, Triton X-100 and then dH$_2$O to volume.

2.   PBS (Dulbecco's Phosphate-Buffered Saline, Gibco Catalog # 450-1300EB).

3.   Blocking Buffer.

4.   Kinase Buffer.

Reagent	M.W.	10x Stock Concentration	1x Working Concentration
HEPES (pH 7.2)	238.3	500 mM	50 mM
MnCl$_2$		20 mM	2 mM
MgCl$_2$	203.32	200 mM	10 mM
Triton-X-100		1 %	0.1%
DTT	380.35	5 mM	0.5 mM

5.   Phenylmethylsulfonyl fluoride (PMSF, Sigma, Cat. No. P-7626):

Working solution: 100 mM in ethanol.

6.   ATP (Bacterial source, Sigma Cat. No. A-7699)

Use 3.31 mg per ml MilliQ H_2O for a stock concentration of 6 mM.

7.   Biotin conjugated anti-phosphotyrosine mab (clone 4G10, Upstate Biotechnology Inc. Cat. No. 16-103, Ser. No. 14495).

8.   Vectastain Elite ABC reagent (Avidin peroxidase conjugate, Vector Laboratories Cat. No. PK-6 100).

9.   ABTS Solution.

10.   Hydrogen peroxide 30% solution (Fisher Catalog # H325).

11.   ABTS/H_2O_2.

12.   0.2 M HCl.

13.   TRIS HCl (Fischer Cat. No. BP 152-5).

Prepare 1.0 mM solution in MilliQ H_2O, adjust pH to 7.2 with HCl.

14.   NaCl (Fisher Cat. No. S271-10).

Prepare 5 M solution in MilliQ H_2O.

15.   MgCl$_2$ (Fisher Cat. No. M33-500).

Prepare 1 M solution in MilliQ H_2O.

16.   HEPES (Fisher Cat. No. BP310-500).

Prepare 1 M solution in MilliQ H_2O, adjust pH to 7.5, sterile filter.

17.   TBST Buffer.

18.   Sodium Carbonate Buffer (Fisher Cat. No. S495).

Prepare 0.1 M solution in MilliQ H_2O, adjust pH to 9.6 with NaOH, filter.

19.   Dithiothreitol (DTT, Fisher Cat. No. BP172-25).

Prepare 0.5 mM working solution in MilliQ H_2O just prior to use. Store at -20° C until used, discard any leftover.

20. $MnCl_2$.

21. Triton X-100.

22. Goat α-Rabbit IgG (Cappel).

23. Affinity purified Rabbit α GST GyrB (Biochemistry Lab. SUGEN, Inc.).

Procedure

All of the following steps are conducted at room temperature unless otherwise indicated.

1. Coat Corning 96-well ELISA plates with 2 μg Goat α-Rabbit antibody per well in Carbonate Buffer such that total well volume is 100 μl. Store overnight at 4° C.

2. Remove unbound Goat a-Rabbit antibody by inverting plate to remove liquid. Pat plate on a paper towel to remove excess liquid and bubbles

3. Add 150 μl Blocking Buffer (5% Low Fat Milk in PBS) to each well. Incubate while shaking on a micro-titer plate shaker for 30 min.

4. Wash 4x with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

5. Add 0.5 μg Rabbit a-GyrB antibody per well. Dilute antibody in DPBS to a final volume of 100 μl per well. Incubate with shaking on a micro-titer plate shaker at room temperature for 1 hour.

6. Wash 4x with TBST as described in step 4.

7. Add 2 μg COS/FGFR cell lysate (Myc-GyrB-FGFR source) in HNTG to each well to give a final volume of 100 μl per well. Incubate with shaking on a micro-titer plate shaker for 1 hour.

8. Wash 4X with TBST as described in step 4.

9.   Add 80 μl of 1x kinase buffer per well.

10.   Dilute test compound 1:10 in 1x kinase buffer + 1% DMSO in a polypropylene 96 well plate.

11.   Transfer 10 μl of diluted test compound solution and control wells from polypropylene plate wells to the corresponding ELISA plate wells, incubate with shaking on a micro-titer plate shaker for 20 minutes.

12.   Add 10 μl of 70 μM ATP diluted in kinase buffer to positive control and test wells (Final ATP concentration is 7 μM/well). Add 10 μl 1x kinase buffer to negative control wells. Incubate with shaking on a micro-titer plate shaker for 15 min.

13.   Stop kinase reaction by adding 5 μl 0.5 M EDTA to all wells.

14.   Wash 4x with TBST as described in step 4.

15.   Add 100 μl biotin conjugated α-phosphotyrosine mab (b4G10) diluted in TBST to each well. Incubate with shaking on a micro-titer plate shaker for 30 minutes.

16.   Prepare Vectastain ABC reagent. Add 1 drop reagent A to 15 ml TBST. Mix by inverting tube several times. Add 1 drop reagent B and mix again.

17.   Wash 4x with TBST as described in step 4.

18.   Add 100 μl ABC HRP reagent to each well. Incubate with shaking on a micro-titer plate shaker for 30 minutes.

19.   Wash 4x with TBST as described in step 4.

20.   Add 100 μl of ABTS/H_2O_2 solution to each well.

22.   Incubate 5 to 15 minutes with shaking. Remove any bubbles.

23.   If necessary stop reaction by adding 1 00 μl of 0.2M HCl/well.

24.   Read assay on Dynatech MR7000 ELISA Plate Reader, test filter: 410 nM, reference filter: 630 nM.

Biochemical FLK-1 Assay

This assay evaluates flk-1 autophosphorylation activity _in vitro_ using ELISA.

Materials And Reagents

5   1.   15 cm tissue culture dishes

         2.   Flk-1/NIH cells: NIH fibroblast line over-expressing human flk-1 clone 3 (SUGEN, Inc., obtained from MPI, Martinsried, Germany).

         3.   Growth medium: DMEM plus heat inactivated 10% FBS

10   and 2 mM Glutamine (Gibco-BRL).

         4.   Starvation medium: DMEM plus 0.5% heat-inactivated FBS, 2 mM Glutamine (Gibco-BRL).

         5.   Corning 96-well ELISA plates (Corning Cat. No. 25805-96).

15   6.   L4 or E38 monoclonal antibody specific for flk-1, Purified by Protein-A agarose affinity chromatography (SUGEN, Inc.).

         7.   PBS (Dulbecco's Phosphate-Buffered Saline) Gibco Cat. No. 450-1300EB).

20   8.   HNTG (see BIOCHEMICAL FGFR for preparation).

         9.   Pierce BCA protein determination kit.

         10.   Blocking buffer

         11.   TBST (pH 7.0)

         12.   Kinase Buffer

25   13.   Kinase Stop Solution: 200 mM EDTA.

         14.   Biotinylated 4G10, specific for phosphotyrosine (UBI, Cat. No. No. 16-103).

         15.   AB kit (Vector Laboratories Cat. No. PK 4000).

         16.   DMSO

30   17.   NUNC 96-well V bottom polypropylene plates (Applied Scientific Cat. No. AS-72092).

         18.   Turbo-TMB (Pierce).

         19.   Turbo-TMB stop solution: 1 M H_2SO_4.

         20.   ATP (Sigma Cat. No. A-7699).

21. 20% DMSO in TBS (pH 7.0).

Procedure

Cell Growth and Lysate Preparation.

1. Seed cell into growth medium and grow for 2-3 days

5   to 90-100% confluency at 37° C and 5% CO_2. Do not exceed

passage #20.

2. Remove the medium and wash the cells twice with PBS.

Lyse with HNTG lysis buffer. Collect all lysates and vortex

mix them for 20-30 seconds.

10 3. Remove insoluble material by centrifugation (5-10

min at about 10,000 xg).

4. Determine the protein concentration using BCA kit.

5. Partition lysate into 1 mg aliquots, store at -80° C.

Assay Procedure

15 1. Coat Corning 96-well ELISA plates with 2 μg/well

purified L4 (or E 38) in 100 μl of PBS. Store overnight at 4° C.

2. Remove unbound proteins from wells by inverting the

plate to remove the liquid. Wash one time with dH_2O, pat plate

on paper towel to remove excess liquid.

20 3. Block plates with 150 μl blocking buffer per well.

Incubate for 45-60 minutes with shaking at 4° C.

4. Remove the blocking buffer and wash the ELISA plate

three times with dH_2O and one time with TBST. Pat plate on

paper towel to remove excess liquid.

25 5. Dilute lysate in PBS to give final concentration of

50 μg/100 μl. Add 100 μl of diluted lysate to each well.

Incubate with shaking at 4° C overnight.

6. Remove unbound proteins from wells by inverting the

plate. Wash as in step 4.

30 7. Add 80 μl of kinase buffer to wells (90 μl to

negative control wells).

8. Dilute test compounds (normally 10-fold) into wells

of a polypropylene plate containing 20% DMSO in TBS.

9. Add 10 µl of the diluted compounds to the ELISA wells containing immobilized flk-1 and shake. Control wells receive no compounds.

10. From stock 1 mM ATP, prepare 0.3 mM ATP solution in dH₂0 (alternatively, kinase buffer may be used).

11. Add 10 µl of 0.3 mM ATP to all wells except the negative controls. Incubate for 60 min. at room temperature with shaking.

12. After 1 hr stop the kinase reaction by adding 11 µl 200 mM EDTA. Shake for 1-2 min.

13. Wash the ELISA plate 4 times with dH₂0 and twice with TBST.

14. Add 100 µl of 1:5000 biotinylated 4G10:TBST to all wells. Incubate 45 min with shaking at room temperature.

15. While the above is incubating, add 50 µl of solutions A & B from the ABC kit to 10 ml of TBST. These solutions must be combined approximately 30 min prior to use.

16. Wash plates as in step 4.

17. Add 100 µl of the preformed A & B complex to all wells. Incubate 30 min with shaking at room temperature.

18. Wash plates as in step 4.

19. Add 100 µl turbo-TMB. Shake at room temperature for 10- 15 min.

20. When the color in the positive control wells reaches an absorbance of about 0.35 - 0.4, stop the reaction with 100 µl of turbo-TMB stop solution.

21. Read plates on Dynatech MR7000 ELISA reader, test filter: 450 nM, reference filter: 410 nM.

HUV-EC-C Assay

The following protocol may also be used to measure a compound's activity against PDGF-R, FGF-R, VEGF, aFGF or Flk-1/KDR, all of which are naturally expressed by HUV-EC cells.

DAY 0

1.   Wash and trypsinize HUV-EC-C cells (human umbilical
vein endothelial cells, (American Type Culture Collection,
catalogue no. 1730 CRL). Wash with Dulbecco's phosphate-
5   buffered saline (D-PBS, obtained from Gibco BRL, catalogue no.
14190-029) 2 times at about 1 ml/10 cm² of tissue culture
flask. Trypsinize with 0.05% trypsin-EDTA in non-enzymatic
cell dissociation solution (Sigma Chemical Company, catalogue
no. C-1544). The 0.05% trypsin is made by diluting 0.25%
10   trypsin/1 mM EDTA (Gibco, catalogue no. 25200-049) in the cell
dissociation solution. Trypsinize with about 1 ml/25-30 cm² of
tissue culture flask for about 5 minutes at 37°C. After cells
have detached from the flask, add an equal volume of assay
medium and transfer to a 50 ml sterile centrifuge tube (Fisher
15   Scientific, catalogue no. 05-539-6).

2.   Wash the cells with about 35 ml assay medium in the
50 ml sterile centrifuge tube by adding the assay medium,
centrifuge for 10 minutes at approximately 200x g, aspirate
the supernatant, and resuspend with 35 ml D-PBS. Repeat the
20   wash two more times with D-PBS, resuspend the cells in about 1
ml assay medium/15 cm² of tissue culture flask. Assay medium
consists of F12K medium (Gibco BRL, catalogue no. 21127-014)
and 0.5% heat-inactivated fetal bovine serum. Count the cells
with a Coulter Counter® (Coulter Electronics, Inc.) and add
25   assay medium to the cells to obtain a concentration of 0.8-1.0
x 10⁵ cells/ml.

3.   Add cells to 96-well flat-bottom plates at 100
µl/well or 0.8-1.0 x 10⁴ cells/well, incubate ~24h at 37°C, 5%
CO₂.

30   **DAY 1**

1.   Make up two-fold test compound titrations in
separate 96-well plates, generally 50 µM on down to 0 µM. Use
the same assay medium as mentioned in day 0, step 2 above.

Titrations are made by adding 90 μl/well of test compound at 200 μM (4X the final well concentration) to the top well of a particular plate column. Since the stock test compound is usually 20 mM in DMSO, the 200 μM drug concentration contains
5   2% DMSO.

A diluent made up to 2% DMSO in assay medium (F12K + 0.5% fetal bovine serum) is used as diluent for the test compound titrations in order to dilute the test compound but keep the DMSO concentration constant. Add this diluent to the
10   remaining wells in the column at 60 μl/well. Take 60 μl from the 120 μl of 200 μM test compound dilution in the top well of the column and mix with the 60 μl in the second well of the column. Take 60 μl from this well and mix with the 60 μl in the third well of the column, and so on until two-fold
15   titrations are completed. When the next-to-the-last well is mixed, take 60 μl of the 120 μl in this well and discard it. Leave the last well with 60 μl of DMSO/media diluent as a non-test compound-containing control. Make 9 columns of titrated test compound, enough for triplicate wells each for: (1) VEGF
20   (obtained from Pepro Tech Inc., catalogue no. 100-200, (2) endothelial cell growth factor (ECGF) (also known as acidic fibroblast growth factor, or aFGF) (obtained from Boehringer Mannheim Biochemica, catalogue no. 1439 600), or, (3) human PDGF B/B (1276-956, Boehringer Mannheim, Germany) and assay
25   media control. ECGF comes as a preparation with sodium heparin.

2.   Transfer 50 μl/well of the test compound dilutions to the 96-well assay plates containing the 0.8-1.0x10^4 cells/100 μl/well of the HUV-EC-C cells from day 0 and
30   incubate ~2 h at 37° C, 5% CO_2.

3.   In triplicate, add 50 μl/well of 80 μg/ml VEGF, 20 ng/ml ECGF, or media control to each test compound condition.

As with the test compounds, the growth factor concentrations
are 4X the desired final concentration. Use the assay media
from day 0 step 2 to make the concentrations of growth
factors. Incubate approximately 24 hours at 37°C, 5% CO_2.

5 Each well will have 50 µl test compound dilution, 50 µl growth
factor or media, and 100 µl cells, which calculates to 200
µl/well total. Thus the 4X concentrations of test compound
and growth factors become 1X once everything has been added to
the wells.

10 **DAY 2**

1. Add ^{3}H-thymidine (Amersham, catalogue no. TRK-686) at
1 µCi/well (10 µl/well of 100 µCi/ml solution made up in RPMI
media + 10% heat-inactivated fetal bovine serum) and incubate
~24 h at 37°C, 5% CO_2. RPMI is obtained from Gibco BRL,

15 catalogue no. 11875-051.

DAY 3

1. Freeze plates overnight at -20°C.

DAY 4

Thaw plates and harvest with a 96-well plate harvester

20 (Tomtec Harvester 96®) onto filter mats (Wallac, catalogue no.
1205-401), read counts on a Wallac Betaplate™ liquid
scintillation counter.

In Vivo Animal Models

Xenograft Animal Models

25 The ability of human tumors to grow as xenografts in
athymic mice (_e.g._, Balb/c, nu/nu) provides a useful _in vivo_
model for studying the biological response to therapies for
human tumors. Since the first successful xenotransplantation
of human tumors into athymic mice, (Rygaard and Povlsen, 1969,

30 _Acta Pathol. Microbial. Scand_. 77:758-760), many different
human tumor cell lines (_e.g._, mammary, lung, genitourinary,
gastro-intestinal, head and neck, glioblastoma, bone, and
malignant melanomas) have been transplanted and successfully

grown in nude mice. The following assays may be used to
determine the level of activity, specificity and effect of the
different compounds of the present invention. Three general
types of assays are useful for evaluating compounds:

5   cellular/catalytic, cellular/biological and in vivo. The
object of the cellular/catalytic assays is to determine the
effect of a compound on the ability of a TK to phosphorylate
tyrosines on a known substrate in a cell. The object of the
cellular/biological assays is to determine the effect of a

10 compound on the biological response stimulated by a TK in a
cell. The object of the in vivo assays is to determine the
effect of a compound in an animal model of a particular
disorder such as cancer.

     Suitable cell lines for subcutaneous xenograft

15 experiments include C6 cells (glioma, ATCC # CCL 107), A375
cells (melanoma, ATCC # CRL 1619), A431 cells (epidermoid
carcinoma, ATCC # CRL 1555), Calu 6 cells (lung, ATCC # HTB
56), PC3 cells (prostate, ATCC # CRL 1435), SKOV3TP5 cells and
NIH 3T3 fibroblasts genetically engineered to overexpress

20 EGFR, PDGFR, IGF-1R or any other test kinase. The following
protocol can be used to perform xenograft experiments:

     Female athymic mice (BALB/c, nu/nu) are obtained from
Simonsen Laboratories (Gilroy, CA). All animals are maintained
under clean-room conditions in Micro-isolator cages with

25 Alpha-dri bedding. They receive sterile rodent chow and water
ad libitum.

     Cell lines are grown in appropriate medium (for example,
MEM, DMEM, Ham's F10, or Ham's F12 plus 5% - 10% fetal bovine
serum (FBS) and 2 mM glutamine (GLN)). All cell culture media,

30 glutamine, and fetal bovine serum are purchased from Gibco
Life Technologies (Grand Island, NY) unless otherwise
specified. All cells are grown in a humid atmosphere of 90-
95% air and 5-10% CO_2 at 37°C. All cell lines are routinely
subcultured twice a week and are negative for mycoplasma as

determined by the Mycotect method (Gibco).

Cells are harvested at or near confluency with 0.05% Trypsin-EDTA and pelleted at 450 x g for 10 min. Pellets are resuspended in sterile PBS or media (without FBS) to a

5   particular concentration and the cells are implanted into the hindflank of the mice (8 - 10 mice per group, 2 - 10 x 10^6 cells/animal). Tumor growth is measured over 3 to 6 weeks using venier calipers. Tumor volumes are calculated as a product of length x width x height unless otherwise indicated.

10   P values are calculated using the Students t-test. Test compounds in 50 - 100 μL excipient (DMSO, or VPD:D5W) can be delivered by IP injection at different concentrations generally starting at day one after implantation.

Tumor Invasion Model

15   The following tumor invasion model has been developed and may be used for the evaluation of therapeutic value and efficacy of the compounds identified to selectively inhibit KDR/FLK-1 receptor.

Procedure

20   8 week old nude mice (female) (Simonsen Inc.) are used as experimental animals. Implantation of tumor cells can be performed in a laminar flow hood. For anesthesia, Xylazine/Ketamine Cocktail (100 mg/kg ketamine and 5 mg/kg Xylazine) are administered intraperitoneally. A midline

25   incision is done to expose the abdominal cavity (approximately 1.5 cm in length) to inject 10^7 tumor cells in a volume of 100 μl medium. The cells are injected either into the duodenal lobe of the pancreas or under the serosa of the colon. The peritoneum and muscles are closed with a

30   6-0 silk continuous suture and the skin is closed by using wound clips. Animals are observed daily.

Analysis

After 2-6 weeks, depending on gross observations of the animals, the mice are sacrificed, and the local tumor metastases to various organs (lung, liver, brain, stomach,

5   spleen, heart, muscle) are excised and analyzed (measurement of tumor size, grade of invasion, immunochemistry, _in situ_ hybridization determination, etc.).

Measurement Of Cell Toxicity

Therapeutic compounds should be more potent in inhibiting

10 receptor tyrosine kinase activity than in exerting a cytotoxic effect. A measure of the effectiveness and cell toxicity of a compound can be obtained by determining the therapeutic index, i.e., IC_{50}/LD_{50}. IC_{50}, the dose required to achieve 50% inhibition, can be measured using standard techniques such as those described

15 herein. LD_{50}, the dosage which results in 50% toxicity, can also be measured by standard techniques as well (Mossman, 1983, _J. Immunol. Methods_, 65:55-63), by measuring the amount of LDH released (Korzeniewski and Callewaert, 1983, _J. Immunol. Methods_, 64:313, Decker and Lohmann-Matthes, 1988, _J. Immunol. Methods_,

20 115:61), or by measuring the lethal dose in animal models. Compounds with a large therapeutic index are preferred. The therapeutic index should be greater than 2, preferably at least 10, more preferably at least 50.

B.   Examples - Biological Activity.

25   Examples of the _in vitro_ potency of compounds of this invention are shown in Table 2. The examples shown are not to be construed as limiting the scope of this invention in any manner whatsoever.

30

CONCLUSION

It will be appreciated that the compounds, methods and pharmaceutical compositions of the present invention are

5   effective in modulating PK activity and therefore are expected to be effective as therapeutic agents against RTK, CTK-, and STK-related disorders.

One skilled in the art would also readily appreciate that the present invention is well adapted to carry out the objects

10   and obtain the ends and advantages mentioned, as well as those inherent herein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as

15   limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are defined by the scope of the claims.

It will be readily apparent to one skilled in the art

20   that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in

25   the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably

30   may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two

terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and

5   described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification

10 and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

In addition, where features or aspects of the invention

15 are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. For example, if X is described as selected from the group consisting of bromine, chlorine,

20 and iodine, claims for X being bromine and claims for X being bromine and chlorine are fully described.
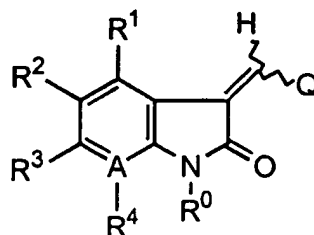
Other embodiments are within the following claims.

CLAIMS

What is claimed:
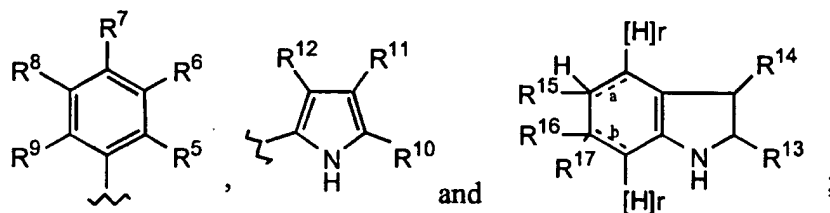
1.   A compound having the chemical structure:

wherein:

A is selected from the group consisting of carbon and nitrogen;

Q is selected from the group consisting of

, and ;

bonds a and b may be either single or double bonds as indicated by the dotted lines with the proviso that a and b are both single bonds or both double bonds in any one compound of this invention;

r is 1 when a and b are double bonds;

r is 2 when a and b are single bonds;

R^0 is selected from the group consisting of hydrogen, alkyl, $-C(O)R^{19}$ and $-C(O)OR^{19}$;

R^1 is selected from the group consisting of hydrogen, alkyl,
alkoxy, halo, aryl, $-(CH_2)_nOC(O)R^{19}$; $-C(O)NR^{19}$; $-C(O)OR^{19}$ and
$-(CH_2)_nR^{20}$, wherein:

  R^{19} is selected from the group consisting of hydrogen,
  alkyl, alkenyl, alkynyl, cycloalkyl and aryl;

  n is 1, 2, 3 or 4;

  R^{20} is selected from the group consisting of hydroxy,
  halo, $-C(O)OR^{19}$, $-OC(O)NR^{21}R^{22}$; $-OC(S)NR^{21}R^{22}$,
  $-OC(O)NHSO_2R^{21}R^{22}$; $-NR^{21}R^{22}$ and a heteroalicylic group
  containing at least one nitrogen atom in the ring, the
  ring being bonded to the adjacent CH_2 group through the
  nitrogen atom;

R^2 is selected from the group consisting of hydrogen, alkyl,
cycloalkyl, aryl, heteroaryl, heteroalicyclic, trihalomethyl,
alkoxy, halo, $-(CH_2)_nR^{20}$, $-SO_2NR^{21}R^{22}$, $-C(O)OR^{19}$, $-C(O)R^{19}$,
$-NHC(O)OR^{19}$, $-NR^{21}R^{22}$ and $-N=CR^{23}$ wherein

  R^{21} and R^{22} are independently selected from the group
  consisting of hydrogen, alkyl, alkenyl, alkynyl,
  cycloalkyl, aryl, heteroaryl, heteroalicyclic;

  R^{23} is selected from the group consisting of alkyl,
  alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl;

R³ is selected from the group consisting of hydrogen, alkyl, trihalomethyl, alkoxy, aryl, aryloxy, heteraryl, heteroalicyclic, hydroxy, halo, $-SO_2NR^{21}R^{22}$, $-NHSO_2R^{19}$, $-C(O)OR^{19}$, $-NR^{21}R^{22}$ and $-NHC(O)R^{24}$, wherein

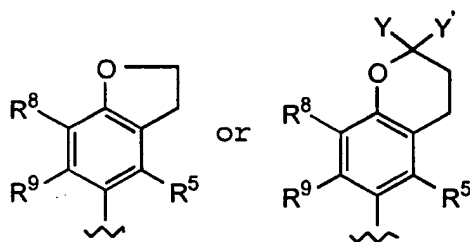R²⁴ is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, heteroaryl and heteroalicyclic;

R⁴ is selected from the group consisting of hydrogen, alkyl, alkoxy and halo;

R⁵ is selected from the group consisting of hydrogen, alkyl, cycloalkyl, halo, aryl and heteroaryl;

R⁶ is selected from the group consisting of hydrogen, alkyl, alkoxy, halo, cycloalkyl, aryl and, combined with R^{18}, a heteroalicyclic group having the structure
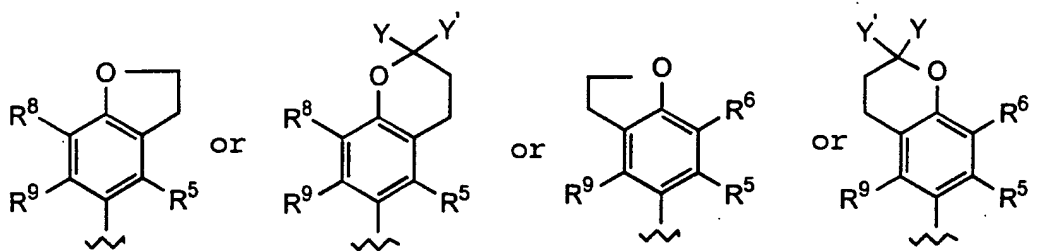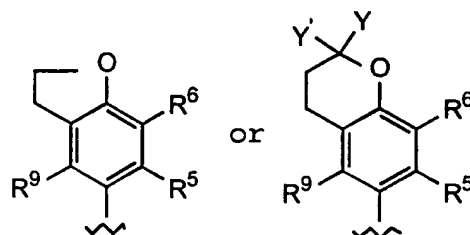

wherein

y and y' are independently selected from the group consisting of hydrogen, alkyl and aryl;

R⁷ is OR^{18}, wherein R^{18} is selected from the group consisting of alkyl, $-(CH)_nR^{20}$ and, combined with R⁶ or R⁸, a heteroalicyclic group having the structure

R^8 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, alkoxy, halo, aryl, heteroaryl and, combined with
5   R^{18}, a heteroalicyclic ring having the structure

R^9 is selected from the group consisting of hydrogen, alkyl, alkoxy, halo and -NR^{21}R^{22};

10

R^{10} is selected from the group consisting of alkyl and -C(O)OR^{19};

R^{11} is selected from the group consisting of hydrogen, alkyl
15   and -C(O)OR^{19};

R^{12} is -(CH$_2$)$_n$R^{20};

R^{13} is either a bond through which Q is bonded to the rest of
20   the molecule or a group selected from the group consisting of hydrogen and alkyl;

R^{14} is either a bond through which Q is bonded to the rest of the molecule or a group selected from the group consisting of hydrogen, alkyl and $-(CH_2)_nR^{20}$;

5   R^{15} is either a bond through which Q is bonded to the rest of the molecule or hydrogen;

         with the proviso that only one of R^{13}, R^{14} or R^{15} is the bond linking Q to the rest of the molecule in any one

10   compound herein;

R^{16} and R^{17}, when a and b are single bonds, are independently selected from the group consisting of hydrogen, alkyl and aryl and, when a and b are double bonds, R^{16} is hydrogen and R^{17} does

15   not exist; and,

physiologically acceptable salts thereof.

         2.   The compound or salt of claim 1, wherein Q is

20
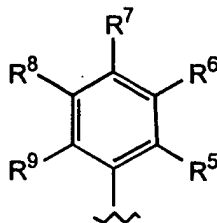
         3.   The compound or salt of claim 2, wherein:

25   R^1 is selected from the group consisting of hydrogen, lower alkyl and $-C(O)OH$;

R^2 is selected from the group consisting of hydrogen, lower alkyl, halo, -C(O)R^{19}, -C(O)OR^{19}, lower alkoxy, -NR^{21}R^{22}, -(CH$_2$)$_n$R^{20} and -NHC(O)OR^{19};

5   R^3 is selected from the group consisting of hydrogen, hydroxy, halo, -NHC(O)O(lower alkyl), -NHSO$_2$R^{19}, -NHC(O)R^{24}, trihalomethyl, and aryl optionally substituted with one or more lower alkoxy groups;

10   R^4 is hydrogen;

R^{19} is selected from the group consisting of hydrogen and lower alkyl;

15   n is 2 or 3;

R^{20} is selected from the group consisting of hydroxy, -C(O)OH, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl, pyrrolidin-1-yl and -NR^{21}R^{22};

20

R^{21} and R^{22} are independently selected from the group consisting of hydrogen and lower alkyl; and,
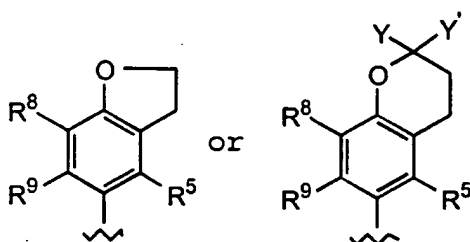
R^{24} is selected from the group consisting of hydrogen and 25   lower alkyl.

     4.   The compound or salt of claim 3, wherein,

R^5 is selected from the group consisting of hydrogen, lower 30   alkyl, halo, five-member ring heteroaryl and aryl optionally substituted with one or more groups selected from the group consisting of lower alkyl, halo, hydroxy, -NR^{21}R^{22} and lower
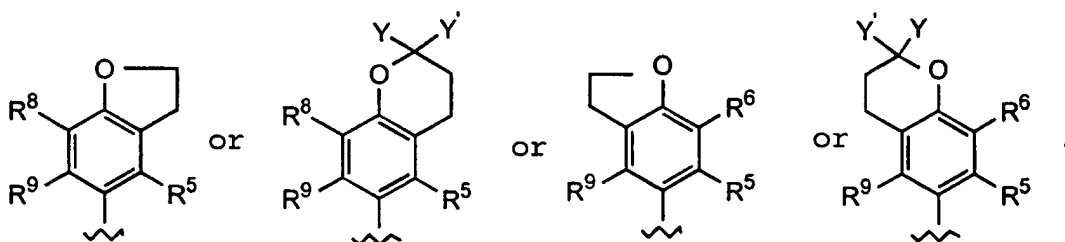
alkoxy;

R[6] is selected from the group consisting of hydrogen, lower
alkyl, 3 to 7-member cycloalkyl, lower alkoxy, halo, aryl

5   optionally substituted with one or more groups independently
selected from the group consisting of lower alkyl, halo,
hydroxy, -NR[21]R[22] and lower alkoxy and, combined with R[18], a
group having the structure

$$ R^8 \underset{R^9}{\overset{O}{\bigcirc}} R^5 \quad or \quad R^8 \underset{R^9}{\overset{Y \quad Y'}{\bigcirc}} R^5 $$

10

wherein y and y' are either both hydrogen or both lower alkyl;

R[7] is -OR[18] wherein R[18] is selected from the group consisting of
lower alkyl, -(CH$_2$)$_n$R[20] and, combined with R[6] or R[8], a group

15   having the structure

$$ R^8 \underset{R^9}{\overset{O}{\bigcirc}} R^5 \quad or \quad R^8 \underset{R^9}{\overset{Y \quad Y'}{\bigcirc}} R^5 \quad or \quad R^9 \underset{}{\overset{O}{\bigcirc}} R^6, R^5 \quad or \quad R^9 \underset{}{\overset{Y' \quad Y}{\bigcirc}} R^6, R^5 \quad ; $$

R[8] is selected from the group consisting of hydrogen, lower
alkyl, 3 to 6-member ring cycloalkyl, lower alkoxy, halo, aryl

20   optionally substituted with one or more groups selected from
the group consisting of lower alkyl, lower alkoxy, halo, -
NR[21]R[22] and -NHC(O)(lower alkyl), five-member heteroaryl having

from 1 to 3 heteroatoms in the ring and 6-member heteroaryl

having from 1 to 3 heteroatoms in the ring and, combined with

R^{18} a group having the structure

or   ;

5   R^9 is selected from the group consisting of hydrogen, lower

alkyl; hydroxy, lower alkoxy, halo and $-NR^{21}R^{22}$.

5.   The compound or salt of claim 3, wherein:

10   R^5 is selected from the group consisting of hydrogen, lower

alkyl, thien-2-yl, thien-3-yl and aryl optionally substituted

with one or more lower alkoxy groups;

R^6 is selected from the group consisting of hydrogen, lower

15   alkyl, 5 or 6-member cycloalkyl, aryl optionally substituted

with one or more lower alkoxy groups and, combined with R^{18}, a

group having the structure

or
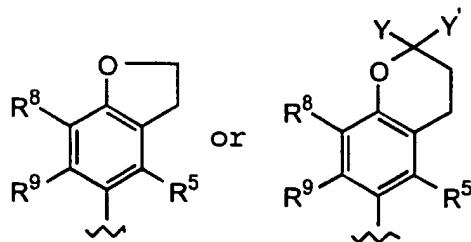
wherein

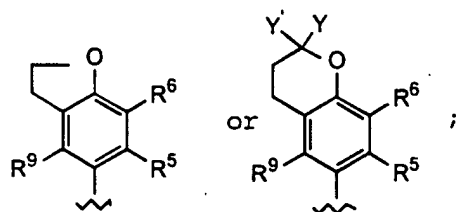20   y and y' are either both hydrogen or both lower alkyl;

R^7 is $-OR^{18}$, wherein R^{18} is selected from the group consisting of lower alkyl, $-(CH_2)_n R^{20}$ and, combined with R^6 or R^8, a group having the structure

5   n is 2 or 3;

R^{20} is selected from the group consisting of hydroxy, $-C(O)OH$, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl pyrrolidin-1-yl and $-NR^{21}R^{22}$;

10

R^8 is selected from the group consisting of hydrogen, lower alkyl, 5 or 6-member ring cycloalkyl, lower alkoxy, aryl optionally substituted with a $-NHC(O)$ (lower alkyl) group, thien-2-yl, thien-3-yl, pyridin-2-yl, pyridin-3-yl and,
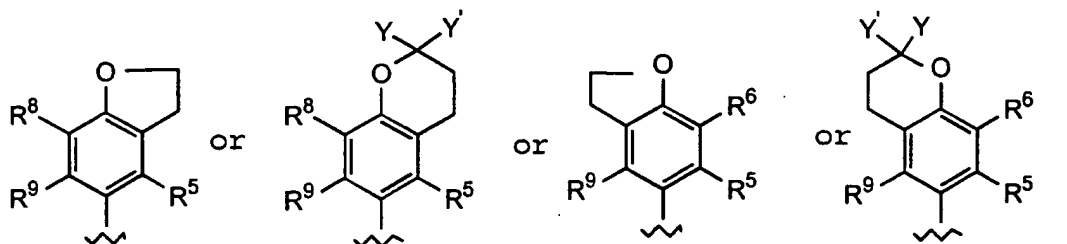
15   combined with R^{18}, a group having the structure

; and
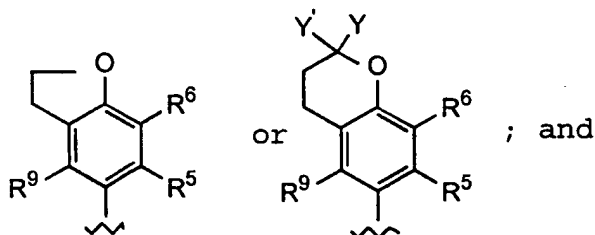
R^9 is selected from the group consisting of hydrogen and lower alkyl.

20

6.   The compound or salt of claim 1 wherein, Q is:
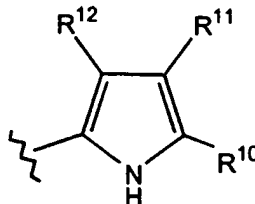
7.   The compound or salt of claim 6, wherein:

5

R^1 is selected from the group consisting of hydrogen, lower
alkyl and $-(CH_2)_nR^{20}$;

n is 2 or 3;

10

R^{20} is selected from the group consisting of hydroxy and
$-C(O)OH$;

R^2 is selected from the group consisting of hydrogen, halo,
15   lower alkyl, lower alkoxy, $-SO_2NR^{21}R^{22}$ and $-C(O)OH$;

R^3 is selected from the group consisting of hydrogen, halo,
lower alkyl, lower alkoxy and aryl optionally substituted with
one or more lower alkoxy groups; and,

20

R^4 is hydrogen.

8.   The compound or salt of claim 7, wherein:

25   R^{10} is selected from the group consisting of lower alkyl and
$-C(O)OR^{19}$;

R^{11} is selected from the group consisting of hydrogen, lower alkyl and $-C(O)OR^{19}$;

R^{12} is $-(CH_2)_nR^{20}$;

R^{19} is selected from the group consisting of hydrogen and lower alkyl;

n is 2 or 3; and,

R^{20} is selected from the group consisting of hydroxy, $-C(O)OH$, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl, pyrrolidin-1-yl and $-NR^{21}R^{22}$.

9.   The compound or salt of claim 7, wherein:

R^{10} is selected from the group consisting of lower alkyl and $-C(O)O(lower\ alkyl)$;

R^{11} is selected from the group consisting of hydrogen, lower alkyl and $-C(O)OR^{19}$;

R^{12} is $-(CH_2)_nR^{20}$;

R^{19} is selected from the group consisting of hydrogen and lower alkyl;

n is 2; and,

R^{20} is selected from the group consisting of $-C(O)OH$, morpholin-1-yl, piperidin-1-yl, piperazin-1-yl, pyrrolidin-1-yl and $-N(lower\ alkyl)_2$.

10. The compound or salt of claim 1, wherein:
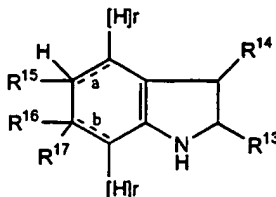
Q is

5   R^1 is selected from the group consisting of hydrogen and lower alkyl;

R^2 is selected from the group consisting of hydrogen, lower alkyl, halo, $-C(O)OR^{19}$, $-C(O)R^{19}$, $-NR^{21}R^{22}$, $-SO_2NR^{21}R^{22}$ and

10   $-N=C-R^{23}$;

R^3 is selected from the group consisting of hydrogen, halo, lower alkyl, lower alkoxy, morpholin-4-yl, piperidin-1-yl, piperazin-1-yl, pyrrolidin-1-yl and aryl optionally

15   substituted with one or more lower alkoxy groups,; and,

R^4 is hydrogen.

11. The compound or salt of claim 10, wherein,

20

a and b are both single bonds;

r is 2;

25   R^{13} is a covalent bond through which Q is bonded to the rest of the molecule;

R^{14} is selected from the group consisting of hydrogen, lower alkyl and -(CH$_2$)$_n$R^{20};

R^{15} is hydrogen;

5

R^{16} and R^{17} are independently selected from the group consisting of hydrogen and lower alkyl.

10   12.   The compound or salt of claim 11 wherein R^{20} is -NR^{21}R^{22} wherein R^{21} and R^{22} are independently selected from the group consisting of hydrogen and lower alkyl.

          13.   The compound or salt of claim 10, wherein,

15

a and b are both double bonds;

r is 1;

20   R^{13} is a covalent bond through which Q is bonded to the rest of the molecule;

R^{14} is selected from the group consisting of hydrogen, lower alkyl and -(CH$_2$)$_n$R^{20};

25

R^{15} and R^{16} are hydrogen; and,

R^{17} does not exist.

30   14.   The compound or salt of claim 10, wherein:

a and b are both double bonds;

r is 1;

R^{13} is selected from the group consisting of hydrogen and lower

5 alkyl;

R^{14} is a covalent bond through which Q is bonded to the rest of the molecule;

10 R^{15} and R^{16} are hydrogen; and,

R^{17} does not exist.

15. The compound or salt of claim 10, wherein:

15

a and b are double bonds;

r is 1;

20 R^{13} is selected from the group consisting of hydrogen and lower alkyl;

R^{14} is selected from the group consisting of hydrogen, lower alkyl and $-(CH_2)_nR^{20}$;

25

R^{15} is a covalent bond through which Q is bonded to the rest of the molecule;

R^{16} is hydrogen; and,

30

R^{17} does not exist.

16. A pharmaceutical composition, comprising

a compound or salt of claim 1; and,

5 a pharmaceutically acceptable carrier or excipient.

17. A method for the modulation of the catalytic
activity of a protein kinase comprising contacting said
protein kinase with a compound or salt of claim 1.

10

18. The method of claim 17 wherein said protein kinase
is selected from the group consisting of a receptor tyrosine
kinase, a non-receptor tyrosine kinase and a serine-
threonine kinase.

15

19. A method for treating or preventing a protein
kinase related disorder in an organism comprising
administering a therapeutically effective amount of a
compound or salt of claim 1 to said organism.

20

20. The method of claim 19 wherein said protein kinase
related disorder is selected from the group consisting of a
receptor tyrosine kinase related disorder, a non-receptor
tyrosine kinase related disorder and a serine-threonine

25 kinase related disorder.

21. The method of claim 19 wherein said protein kinase
related disorder is selected from the group consisting of an
EGFR related disorder, a PDGFR related disorder, an IGFR

30 related disorder and a flk related disorder.

22. The method of claim 19 wherein said protein kinase related disorder is a cancer selected from the group consisting of squamous cell carcinoma, astrocytoma, Kaposi's sarcoma, glioblastoma, lung cancer, bladder cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, small-cell lung cancer, glioma, colorectal cancer, genitourinary cancer and gastrointestinal cancer.

23. The method of claim 19 wherein said protein kinase related disorder is selected from the group consisting of diabetes, an autoimmune disorder, a hyperproliferation disorder, restenosis, fibrosis, psoriasis, von Heppel-Lindau disease, osteoarthritis, rheumatoid arthritis, angiogenesis, an inflammatory disorder, an immunological disorder and a cardiovascular disorder.

24. The method of claim 19 wherein said organism is a human.

25. A compound of claim 1, or salt thereof, selected from the group consisting of:
3-(3,5-Diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
5-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide
3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-hydroxy-1,3-dihydroindol-2-one
5-Acetyl-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one
3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester

3-(3-Isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one

5-Chloro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one

3-(3-Cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-(3-Cyclopentyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one

3-(3-Cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

5-Bromo-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

5-Chloro-3-(2,3-dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one

5-Chloro-3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one

N-{3-[3-Cyclohexyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-methoxy-1,3-dihydroindol-2-one

N-[3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-dihydroindol-2-one

5-Amino-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

5-Chloro-3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-fluoro-1,3-dihydroindol-2-one

3-(2,2-Dimethylchroman-6-ylmethylene)-5-fluoro-1,3-dihydroindol-2-one

5-Chloro-3-[3,5-diisopropyl-4-(2-morpholin-4-ylethoxy)-

benzylidene]-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-7-fluoro-1,3-dihydroindol-2-one

3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-5-(2-morpholin-4-ylethyl)-1,3-dihydroindol-2-one

N-[3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-ethyl-1,3-dihydroindol-2-one

N-[2'-Methoxy-5'-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-biphenyl-3-yl]-acetamide

5-Fluoro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one

N-[3-(4-Methoxy-3-thiophen-2-ylbenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide

6-Amino-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

N-[3-(2,2-Dimethylchroman-6-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-acetamide

5-Bromo-3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one

3-(4-Methoxy-3-thiophen-3-ylbenzylidene)-1,3-dihydroindol-2-one

5-Bromo-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydroindol-2-one

5-Fluoro-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

3-(3-Isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

3-(4,5-Dimethoxy-2-thiophen-2-ylbenzylidene)-1,3-dihydroindol-2-one

N-{3-[4-(2-Morpholin-4-ylethoxy)-3-thiophen-2-ylbenzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

3-(2,2-Dimethylchroman-6-ylmethylene)-4-methyl-1,3-dihydroindol-2-one

3-(2,3-Dihydrobenzofuran-5-ylmethylene)-5-fluoro-1,3-dihydroindol-2-one

3-(3-Cyclohexyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one

5-Fluoro-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

3-(3'-Ethoxy-6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

3-(3-Cyclopentyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

3-(3-Cyclopentyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

3-(4,5,2'-Trimethoxybiphenyl-2-ylmethylene)-1,3-dihydroindol-2-one

N-{3-[4-(2-Morpholin-4-ylethoxy)-3-thiophen-3-ylbenzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

5-Chloro-3-(3-cyclohexyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-carbamic acid tert-butyl ester

3-(3,5-Diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Bromo-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

N-{3-[3-tert-Butyl-4-(2-morpholin-4-ylethoxy)-benzylidene]-2-oxo-2,3-dihydro-1H-indol-6-yl}-acetamide

3-(4-Methoxy-3,5-dimethylbenzylidene)-1,3-dihydropyrrolo[2,3-b]pyridin-2-one

5-Bromo-3-[3,5-diisopropyl-4-(2-morpholin-4-ylethoxy)-
benzylidene]-1,3-dihydroindol-2-one

3-(3'-Ethoxy-4,5-dimethoxybiphenyl-2-ylmethylene)-1,3-
dihydroindol-2-one

5-Chloro-3-(4-methoxy-3-thiophen-2-ylbenzylidene)-1,3-
dihydroindol-2-one

5-Chloro-3-(4-methoxy-3-pyridin-3-ylbenzylidene)-1,3-
dihydroindol-2-one

5-Chloro-3-(4,5,3'-trimethoxybiphenyl-2-ylmethylene)-1,3-
dihydroindol-2-one

3-(4,5-Dimethoxy-2-naphthalen-2-ylbenzylidene)-1,3-
dihydroindol-2-one

N-[3-(3'-Acetylamino-6-methoxybiphenyl-3-ylmethylene)-2-oxo-
2,3-dihydro-1H-indol-6-yl]-acetamide

6-Methoxy-3-(4-methoxy-3-thiophen-3-ylbenzylidene)-1,3-
dihydroindol-2-one

3-(6-Methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-2-one

3-(2,3-Dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one

5-Chloro-3-(6-methoxybiphenyl-3-ylmethylene)-1,3-dihydroindol-
2-one

3-(3-Cyclohexyl-4-methoxybenzylidene)-4-methyl-1,3-
dihydroindol-2-one

3-(2,3-dihydrobenzofuran-5-ylmethylene)-4-methyl-1,3-
dihydroindol-2-one

3-(3-Isopropyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-
b]pyridin-2-one

3-(6-Methoxybiphenyl-3-ylmethylene)-1,3-dihydropyrrolo[2,3-
b]pyridin-2-one

3-(3-Cyclohexyl-4-methoxybenzylidene)-1,3-dihydropyrrolo[2,3-
b]pyridin-2-one

3-(2,3-Dihydrobenzofuran-5-ylmethylene)-1,3-dihydropyrrolo[2,3-
b]pyridin-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-1,3-
dihydropyrrolo[2,3-b]pyridin-2-one

5-Bromo-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-
2-one

5-Bromo-3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-
dihydroindol-2-one

5-Chloro-3-(3-cyclopentyl-4-methoxybenzylidene)-4-methyl-1,3-
dihydroindol-2-one

5-Chloro-3-(6-methoxybiphenyl-3-ylmethylene)-4-methyl-1,3-
dihydroindol-2-one

5-Chloro-3-(5-isopropyl-4-methoxy-2-methylbenzylidene)-4-
methyl-1,3-dihydroindol-2-one

5-Chloro-3-(4-methoxy-3,5-dimethylbenzylidene)-4-methyl-1,3-
dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-trifluoromethyl-1,3-
dihydroindol-2-one

6-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-
dihydroindol-2-one

3-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-
1H-indol-5-yl]-propionic acid

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-methoxy-1,3-
dihydroindol-2-one

5-Butyl-3-(3,5-diisopropyl-4-methoxybenzylidene)-1,3-
dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-
indole-4-carboxylic acid

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-(3-methoxyphenyl)-
1,3-dihydroindol-2-one

7-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-5-methyl-1,3-
dihydroindol-2-one

[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-
indol-5-yl]-carbamic acid tert-butyl ester

5-Chloro-3-(3-isopropyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

5-Chloro-3-(3-cyclopentyl-4-methoxybenzylidene)-1,3-dihydroindol-2-one

3-(6-Methoxybiphenyl-3-ylmethylene)-4-methyl-1,3-dihydroindol-2-one

3-(5-Isopropyl-4-methoxy-2-methylbenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Bromo-3-(2,3-dihydrobenzofuran-5-ylmethylene)-1,3-dihydroindol-2-one

5-Chloro-3-(3-isopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Chloro-3-(3,5-diisopropyl-4-methoxybenzylidene)-4-methyl-1,3-dihydroindol-2-one

5-Chloro-3-(2,2-dimethylchroman-6-ylmethylene)-4-methyl-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5,6-dimethoxy-1,3-dihydroindol-2-one

N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-methanesulfonamide

N-[3-(3,5-Diisopropyl-4-methoxybenzylidene)-2-oxo-2,3-dihydro-1H-indol-6-yl]-benzamide

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-(3-ethoxyphenyl)-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-phenyl-1,3-dihydroindol-2-one

3-(3,5-Diisopropyl-4-methoxybenzylidene)-5-fluoro-1,3-dihydroindol-2-one

5-Fluoro-3-(4-methoxy-3,5-dimethylbenzylidene)-1,3-dihydroindol-2-one

3-(2,2-dimethylchroman-6-ylmethylene)-1,3-dihydroindol-2-one

26. A compound of claim 1, or a physiologically
acceptable salt thereof, selected from the group consisting
of:

5-methyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-

5   dihydroindol-2-one,

3-(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-

indol-5-sulfonic acid amide,

3(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-

indole-5-sulfonic acid methylamide,

10   3-(3-methyl-1H-indole-2-ylmethylene)-2-oxo-2,3-dihydro-1H-

indole-5-sulfonic acid dimethylamide,

3-(3-methyl-1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-

indole-5-carboxylic acid,

5-acetyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-

15   dihydroindol-2-one,

5-acetyl-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

3-(1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indol-5-

sulfonic acid amide,

5-amino-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

20   3-(1H-indol-2-ylmethylene)-2-oxo-2,3-dihydro-1H-indole-5-

carboxylic acid,

6-chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

5-chloro-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

25   5-bromo-3-(1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

3-(1H-indol-2-ylmethylene)-4-methyl-1,3-dihydroindol-2-one,

3-(3-methyl-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one,

5-chloro-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-

dihydroindol-2-one,

30   5-bromo-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-

dihydroindol-2-one,

4-methyl-3-(3-methyl-1H-indol-2-ylmethylene)-1,3-

dihydroindol-2-one, and

3-(1H-indol-2-ylmethylene)-5[(1H-indol-2-ylmethylene)amino]-

5   1,3-dihydroindol-2-one.

27. A compound of claim 1, or a physiologically
acceptable salt thereof, wherein said compound is selected
from the group consisting of:

10   3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-
tetrahydro-1H-indol-3-yl]-propionic acid,
3-[2-(5-chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,
3-2-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-

15   4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,
3-[2-(4-methyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,
3-[2-(5-methyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,

20   3-[2-(6-chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,
3-[2-(6-methoxy-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,
N,N-dimethyl-3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-

25   4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide,
3-[3-(3-dimethylamino-propyl)-4,5,6,7-tetrahydro-1H-indol-2-
ylmethylene]-1,3-dihydro-indol-2-one,
3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-
tetrahydro-1H-indol-3-yl]-propionamide,

30   3-[3-(3-morpholin-4-yl-3-oxo-propyl)-4,5,6,7-tetrahydro-1H-
indol-2-ylmethylene]-1,3-dihydro-indol-2-one,
N-methyl-3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-

4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide,

N-(2-morpholin-4-yl-ethyl)-3-[2-(2-oxo-1,2-dihydro-indol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-
propionamide, 3-[2-(2-oxo-1,2-dihydro-pyrrolo[2,3-b]pyridin-
3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid,

3-{2-[6-(3-methoxy-phenyl)-2-oxo-1,2-dihydro-indol-3-
ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic
acid,

3-{2-[6-(4-methoxy-phenyl)-2-oxo-1,2-dihydro-indol-3-
ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic
acid,

3-[2-(2-oxo-6-phenyl-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid,

3-{2-[6-(2-methoxy-phenyl)-2-oxo-1,2-dihydro-indol-3-
ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic
acid,

3-[2-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid,

3-[2-(6-morpholin-4-yl-2-oxo-1,2-dihydro-indol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid,

3-[2-(5-chloro-4-methyl-2-oxo-1,2-dihydro-indol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid,

3-[2-(5-bromo-4-methyl-2-oxo-1,2-dihydro-indol-3-
ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic
acid,

3-[2-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-N-(2-morpholin-4-yl-
ethyl)-propionamide,

3-[2-(5-chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-
4,5,6,7-tetrahydro-1H-indol-3-yl]-N-(2-morpholin-4-yl-
ethyl)-propionamide, and
3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-phenyl]-

5   propionic acid.

28. A process for the preparation of a 3-(3-
($R^{27}R^{28}N$)propyl)-4,5,6,7-tetrahydro-1H-indole, comprising:
        reacting 5-aminolevulinic acid or its hydrochloride

10   salt with 1,3-cyclohexadione, optionally substituted in the
5-position with one or two groups independently selected
from the group consisting of alkyl and aryl, in a first
solvent in the presence of a base at a temperature of from
about 60° to about 180° C to give an optionally substituted

15   3-(4-oxo-4,5,6,7-tetrahydro-1H-indol-3-yl)propionic acid;
        reacting said 3-(4-oxo-4,5,6,7-tetrahydro-1H-indol-3-
yl)propionic acid with an $R^{27}R^{28}NH$ group in which R^{27} and R^{28}
are independently selected from the group consisting of
hydrogen, alkyl, alkenyl, alkynyl and aryl or, combined, R^{27}

20   and R^{28} form a five-member heteroaryl group with the nitrogen
atom or form a five-member or a six-member heteroalicyclic
group with the nitrogen atom, to give an optionally
substituted 3-(3-($R^{27}R^{28}N$)-3-oxopropyl)-4,5,6,7-tetrahydro-
indol-4-one; and,

25   reducing said 3-(3-($R^{27}R^{28}N$)-3-oxopropyl)-4,5,6,7-
tetrahydroindol-4-one with a metal hydride reducing agent in
a second solvent at a temperature of from about room
temperature to about 80° C to give a 3-(3-$R^{27}R^{28}N$)propyl-
4,5,6,7-tetrahydro-1H-indole.

30

29. The process of claim 28 wherein reacting said 5-
aminolevulinic acid or its hydrochloride salt with said

optionally substituted 1,3-cyclohexadione comprises using
water as said first solvent.

30.   The process of claim 28 wherein said reacting said
5 5-aminolevulinic acid or its hydrochloride salt with said
optionally substituted 1,3-cyclohexadione comprises using
sodium acetate as said base.

31.   The process of claim 28, wherein reacting said 5-
10 aminolevulinic acid or its hydrochloride salt with said
optionally substituted 1,3-cyclohexadione comprises using a
temperature of from about 100° C to about 120° C.

32.   The process of claim 28 comprising using as R^{27} and
15 R^{28} groups independently selected from the group consisting
of lower alkyl and, combined, $-(CH_2)_4-$, $-(CH_2)_5-$, $-CH_2)_2O(CH_2)_2-$
and $-(CH_2)_2N(CH_2)_2-$.

33.   The process of claim 28 in which said reaction of
20 said $R^{27}R^{28}NH$ with said 3-(4-oxo-4,5,6,7-tetrahydro-1H-indol-
3-yl)propionic acid comprises using 1,1-carbonyldiimidazole.

34.   The process of claim 28 in which reducing said 3-
(3-($R^{27}R^{28}N$)-3-oxopropyl)-4,5,6,7-tetrahydroindol-4-one
25 comprises using lithium aluminum hydride.

35.   The process of claim 28 in which reducing said 3-
(3-($R^{27}R^{28}N$)-3-oxopropyl)-4,5,6,7-tetrahydroindol-4-one
comprises using tetrahydrofuran as said second solvent at a
30 temperature of from about room temperature to the reflux
temperature of said tetrahydrofuran.

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(57) Abstract

The present invention relates to novel 3–methylidenyl–2–indolinone compounds and physiologically acceptable salts and prodrugs thereof which modulate the activity of protein kinases and therefore are expected to be useful in the prevention and treatment of protein kinase related cellular disorders such as cancer.

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A. CLASSIFICATION OF SUBJECT MATTER

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B.   FIELDS SEARCHED

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,409,949 A (BUZZETTI et al.) 25 April 1995, See Claim 1 and the abstract.	1-35
A	US 5,650,415 A (TANG et al.) 22 July 1997, see claim 1 and the abstract.	1-35
A	US 5,374,652 A (BUZZETTI et al.) 20 December 1994, see claim 1 and the abstract.	1-35
A	US 5,339,992 A (BARTHOMEUF et al.) 23 August 1994, see claim 1 and the abstract.	1-35
A	US 5,382,593 A (LE BAUT et al.) 17 January 1995, see claim 1 and the abstract.	1-35

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